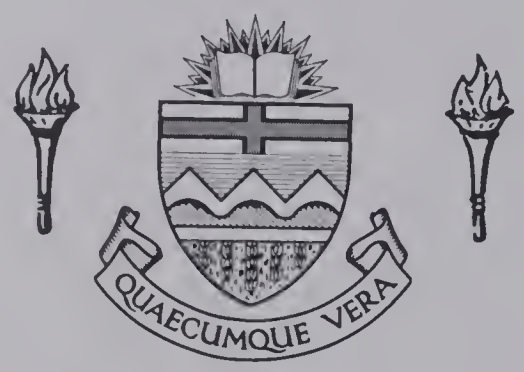

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SEASONAL VARIATIONS IN THE MASS AND COMPOSITION OF BROWN ADIPOSE TISSUE IN THE MEADOW VOLE, *MICROTUS PENNSYLVANICUS*

by

© LARRY ALVIN DIDOW

A THESIS

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OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled Seasonal Variations in the Mass and Composition of Brown Adipose Tissue in the Meadow Vole, *Microtus pennsylvanicus*, submitted by Larry Alvin Didow in partial fulfilment for the degree of Master of Science.

ABSTRACT

Mass of brown adipose tissue was investigated in the meadow vole *(Microtus pennsylvanicus)* collected from its natural habitat throughout a year. Environmental temperature, sex, and total body weight were recorded in an attempt to relate any change in mass of brown adipose tissue to age or environmental change.

Mass of brown adipose tissue relative to body weight showed significant seasonal changes in both mature and immature meadow voles. The seasonal patterns showed an inverse relationship to environmental temperature. In winter, the relative weight of brown fat increased twofold in immature voles and fourfold in mature voles over the levels maintained in summer. Young voles showed a higher amount of brown fat relative to body weight than older voles in summer and early winter. No significant difference existed in the relative weight of brown fat of immature males and females. Mature female voles showed significant differences in the relative weight of brown fat from that of mature male voles in spring and early winter.

Gross chemical composition of brown adipose tissue was investigated to determine whether any seasonal change might occur. Changes in mass of brown adipose tissue were associated with parallel changes in the absolute amount of water, lipid, and protein content.

These findings are interpreted as indicating that brown adipose tissue in the meadow vole is an important site of nonshivering thermogenesis, which responds in mass to the seasonal variations in the need for thermoregulatory heat production.

ACKNOWLEDGEMENTS

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LIST OF TABLES

LIST OF FIGURES

TABLE OF CONTENTS

INTRODUCTION

The physiological adjustments of homeotherms to cold and to climatic changes have received considerable attention in the past two decades (see reviews by Hart, 1963; Jansky, 1965). Many studies have demonstrated that cold-acclimated, small mammals exhibit an increased capacity to produce heat when exposed to low temperatures. Moreover, Héroux et al. (1956), Cottle and Carlson (1956), and Davis et al. (1960) have shown that small mammals, during chronic exposure to cold, decrease the amount of their shivering and consequently the extra heat production is a result of non-shivering or "chemical" thermogenesis. Similarly, it was shown that metabolic acclimatization is associated with the enhancement of non-shivering thermogenesis in wild rats during the winter (Héroux et al., 1959; Héroux, 1962; Hart and Héroux, 1963).

Several workers (Depocas, 1960; Davis, 1963; Jansky et al., 1964) have attempted to discern which tissues or organs are of particular importance as sites of non-shivering heat production. Since 1963 (Smith, 1964; Smalley and Dryer, 1967), considerable evidence has been obtained which implicates brown fat as a tissue capable of producing large quantities of heat for thermoregulation, and that its maximal thermogenic capacity is evoked under such conditions as arousal from hibernation (Hayward and Lyman, 1967), during cold acclimation (Smith and Roberts, 1964), and cold exposure of newborn mammals (Hull and Segall, 1965).

There have been many studies on the dynamics of brown adipose tissue in hibernators and laboratory mammals, but little has been done in ascertaining the temperature adaptive significance of brown fat in

small nonhibernating mammals under natural environmental conditions. Therefore, this study was undertaken to determine if brown fat (as evidenced by its mass and composition) may have a significant role in thermoregulatory heat production in mice under natural conditions.

On the basis of laboratory studies, dramatic changes have been shown to occur in brown adipose tissue during cold exposure and arousal from hibernation. Joel (1965) found that young rats adapted to cold (5°C) increased their mass of brown fat 2.7 times. Similarly, in adult rats, the total mass of brown fat relative to body weight increased 20 - 25% after three hours of cold exposure (6°C) and reached a maximum twofold increase after eight days (Roberts and Smith, 1967a). Roberts and Smith (1967a) found that the total nitrogen content doubled during cold acclimation of the adult rat. The percentage of lipid in brown fat was found to decrease after cold exposure in rats (Cameron and Smith, 1964; Steiner and Cahill, 1964; Joel, 1965) and during arousal from hibernation (Grodums et al., 1966). Under laboratory conditions the seasonal changes in lipid content of brown fat showed a higher level in bats during the hibernating season (Wells et al., 1965).
No seasonal difference, with regard to lipid content in brown fat, was found in the ground squirrel *Citellus lateralis* (Grodums et al., 1966).

Metabolic studies on cold acclimated rats (Smith and Roberts, 1964; Roberts and Smith, 1967b) showed that brown fat cells have a high respiratory rate and that the *in vitro* oxygen consumption of this tissue is less sensitive to a decrease in temperature than any other tissue studied, thus indicating its capacity for heat production. Heim and Hull (1966) calculated that brown adipose tissue in the new-born rabbit uses

over two-thirds of the extra oxygen consumed for thermogenesis during noradrenaline infusion and cold exposure, and conclude that brown fat is the major site of heat production.

Recent studies on brown fat show that noradrenaline is the mediator for non-shivering thermogenesis (see review by Himms-Hagen, 1967). The abundant sympathetic innervation (Wirsen, 1964) and the large number of mitochondria (Napolitano and Fawcett, 1958; Hull, 1966; Hayward and Lyman, 1967) in brown adipose tissue would provide a rapid "on" and "off" mechanism for heat production. Furthermore, brown fat depots have been shown to have a high blood flow rate during cold acclimation (Kuroshima and Konno, 1967), during arousal from hibernation (Bullard and Funkhauser, 1964; Rauch, pers. comm.), and during moderate cold exposure in newborn rabbits (Heim and Hull, 1966).

One of the most important aspects of brown fat is its location. The tissue is "strategically" located around the major blood vessels and nerve tracts supplying the cervical and thoracic regions (Cameron and Smith, 1964; Smith and Roberts, 1964; Rauch, pers. comm.). Brown fat has been found in most mammalian embryos including man, and is retained after birth in all true hibernators and several small mammals (see review by Johansson, 1959).

In view of the evidence obtained from laboratory studies, the mass and location, composition, innervation, structure, and response rates of brown adipose tissue show that it has the potential for rapid heat production during changes in environmental temperature. However, little evidence has been obtained on the dynamics of brown adipose tissue in a natural population of small rodents, in which changes in the mass and

composition of the tissue may be expected to result from changes in seasonal temperature conditions. Therefore, the purpose of this project is to study the seasonal changes in mass and composition, as well as the structure, of brown fat in the meadow vole *Microtus pennsylvanicus*, in order to ascertain its temperature-adaptive significance. This study was designed to sample large numbers of a species of small mammal in which adaptations to extreme temperature conditions are essential for survival. Thus, the meadow vole was chosen for this study because of its abundance in the area and its continuous activity throughout the year.

METHODS

DESCRIPTION OF HABITAT

All the meadow voles *(Microtus pennsylvanicus)* were collected within a 50 mile radius of Edmonton, Alberta (Lat. 53°,30'N; Long. 113°,45'W). This aspen parkland is primarily under cultivation and provides a suitable habitat for the meadow vole. In the spring and summer of 1966, a large population of *M. pennsylvanicus* occurred in this region. They were collected along roadways and fencelines surrounding grain and hay fields. In fall and winter, the voles were obtained from hay bales, swathed grain, and heavily vegetated marsh areas.

Environmental temperature data were obtained from two sources. The mean monthly temperatures of the Edmonton area were calculated from the official records of mean daily temperatures at the Edmonton International Airport. These data were obtained from the Meteorological Branch of the Department of Transport during the period of this study. Temperatures of the microhabitat were recorded periodically throughout the year by using thermistors and a telethermometer.[1] Temperatures were measured along runways, in burrows, under hay bales, and beneath the snow.

SAMPLING PROCEDURES

Meadow voles were collected each month from their natural habitat during the period April 1966 to April 1967. One hundred voles were captured at the beginning of each month with the exception of January, February, and March during which a total of 120 animals were obtained by

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[1]Yellow Springs Instrument Co. Model 42SC.

continuous sampling. During this period the population number was low and the presence of snow hindered the collection of large samples of voles.

From April to January, all meadow voles were captured by hand. This method of sampling was not dependent upon the spontaneous activity of the meadow vole; therefore, it enabled one to obtain a representative sample of the population with respect to age and sex. Large numbers of animals were caught over a short period of time each month and very young mice were sampled before they matured. Live animals were returned to the University laboratory within 1 - 3 hours of capture, and were killed immediately in an ether chamber. They were then placed in plastic bags and stored at -25°C to minimize weight loss and any possible chemical changes. In February and March snap traps were set every week under the snow where meadow vole activity was evident. The traps were checked twice a day. The use of snap traps provided a method of killing the animals and allowing the ambient temperature to immediately freeze them.

With respect to this study, the most important feature of the sampling procedures is that the time from capture to killing and preservation was minimized. Smalley and Dryer (1967) state that small differences in environmental conditions could lead to pronounced differences in the chemical composition of brown fat. Such factors as diet and temperature may affect body weights, as well as mass and composition of brown fat. Furthermore, Rauch (pers. comm.) noticed that brown fat from wild-caught animals became paler in color under laboratory conditions.

AGEING OF MICE

Previous studies (Dawkins and Hull, 1964; Buchalczyk and Korybska,

1964) have shown that the mass of brown adipose tissue varies with age of the animal. Therefore, all voles used in this study were placed into two categories: mature and immature. This distinction was determined by the total body weight and an examination of the reproductive organs of each individual. Criteria of maturity in females were presence of developing follicles in the ovaries, large size of the uterus, or the presence of placental scars. The number of developing embryos and placental scars was recorded in order to estimate the reproductive potential of *M. pennsylvanicus* during that year. Males were considered mature if tubules were visible in the epididymis. In doubtful cases smears of testis were made and observed for the presence of sperm. The above observations do not provide an accurate estimation of age but only a distinction between those individuals which are sexually mature and those which are not.

Total body weights of frozen mice were taken throughout the entire study and were used for calculations. Little difference existed between the body weights of freshly killed, frozen, and thawed animals (<0.2g).

DISSECTION OF TOTAL BROWN ADIPOSE TISSUE

Thirty to fifty voles each month were analyzed for the total amount of brown adipose tissue. The brown fat was carefully removed from each individual and placed in a tared snap-cap vial containing a small piece of sponge and some paraffin oil. This method was used to prevent desiccation of the tissue during the time involved to complete the dissection. Care was taken to remove any major blood vessels surrounded by brown fat deposits and any white fat surrounding the tissue. The tissue was weighed to the nearest milligram. It was very difficult to

remove all the brown adipose tissue overlying the dorsal aorta and thoracic vertebrae. Although a small amount of brown fat was left in this region, it was estimated that approximately 95 - 98% of the total brown fat was removed.

After weighing, the brown adipose tissue (which had been placed in paraffin oil) was not used for any further analysis, and was discarded. However, in January, February, and March the sample sizes of voles were small and the same tissue was used for the determination of gross composition, as well as mass. In these cases, the large deposits were removed, weighed immediately, and prepared for chemical analysis. The remaining deposits were removed and placed in paraffin oil as previously described. The total mass of tissue was then determined by addition of the two tissue weights.

DETERMINATION OF GROSS CHEMICAL COMPOSITION OF BROWN FAT

a. Total Water Content

The large deposits of brown fat surrounding the neck region were used for determining the composition of this tissue. Each sample consisted of tissue from five voles of the same sex and approximately the same body weight. Brown fat was removed, minced with a sharp blade and placed in a tared weighing bottle. The tissue was weighed to the nearest milligram and then dried to a constant weight in a freeze dryer[2] for 72 hours. The water content was determined by the difference between the wet and dry weights of the tissue.

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[2]L-Thermovac Model FD-3.

b. Lipid Content

The dried tissue was transferred quantitatively to a tared 10 x 50 mm extraction thimble and inserted into a Soxhlet micro-extraction apparatus. Fat was extracted with petroleum ether (b.p.30-60°C) for 24 hours. Samples were then oven dried at 75°C for 24 hours and the decrease in weight of the tissue was recorded as the weight of ether-extractable lipid. Homogeneous samples of beef liver were prepared, dried, and extracted by the methods described, and were used as control standards for the analysis technique.

c. Nitrogen Content

The fat free, dry tissue in the extraction thimble was then transferred to a 30ml digestion flask containing concentrated H_2SO_4, K_2SO_4, and mercuric oxide as the catalyst. The samples were hydrolyzed under low heat until a clear solution formed and then the contents were diluted to 100 ml. Duplicate 25ml aliquots of the digestion mixture were distilled in a micro-kjeldhal distillation apparatus with 20mls of strong base solution (50g NaOH + 5g $Na_2S_2O_3.5H_2O$ in 100mls). The ammonia gas released was trapped in 4% boric acid containing four drops of methyl red-bromocresol green indicator (5 parts 0.2% bromocresol green + 1 part 0.2% methyl red in ethanol). The ammonium borate formed was titrated with standardized 0.12N HCl. The percentage of nitrogen present in the mixture was calculated according to the formula:

$$\%N = \frac{4(T-B)NE100}{W}$$

where T equals the mls of titre, B equals the blank, N equals the normality of the acid, E equals the equivalent weight of nitrogen, and W equals the

weight of the total sample in mg. Urea was used to standardize the analysis technique, and the fat extraction thimbles were examined for the presence of nitrogen.

HISTOLOGY OF BROWN ADIPOSE TISSUE

a. Paraffin Sections

Preliminary histological examination of suspected brown fat deposits was conducted as a means of positive identification of the tissue. The tissue was fixed for one hour in Zenker's fixative (Baker, 1963), washed for one hour, dehydrated, and embedded in paraffin. Eight μ-sections were cut and stained with Ehrlich's haemotoxylin and eosin.

b. Epon Sections

For fine structure of brown adipose tissue, electron microscopy was used. One to two millimeter pieces of interscapular brown fat were fixed in 3% gluteraldehyde, washed in 10% sucrose, and post-fixed in 2% osmium tetroxide. All the above solutions were made up in 0.1M phosphate buffer at pH 7.3, and each step was carried out at 4°C for two hours. The tissue was then washed in distilled water, dehydrated in a graded series of ethanols as follows: 30%, 50%, 70%, 80%, and 90% for 10 minutes each, and four 10 minute changes in 100% ethanol. Following two changes of 5 minutes each in propylene oxide, the tissue was passed into the following mixture:

7 parts resin A (Epon 812 - DDSA)
3 parts resin B (Epon 812 - MNA)
2%v/v DMP-30 accelerator (2,4,6,triphenol)
10 parts propylene oxide.

The tissue was allowed to stand in the above mixture overnight in an

uncovered vial to permit ready evaporation of the propylene oxide. The tissue was then embedded in a fresh mixture of Epon containing no propylene oxide, and polymerized for 24 hours at 60°C.

All sections were cut with glass knives on a Porter-Blum (MT-1) microtome at thicknesses varying from 600 A (silver-gray) to 1000 A (gold). Thin sections were mounted on Formvar-coated 200-mesh copper grids. Mounted thin sections were stained with 2% uranyl acetate in methanol (Stempak and Ward, 1964) for 3 minutes, rinsed in three changes of distilled water, and counterstained with 0.3% lead citrate (Venable and Coggeshall, 1965), followed by three rinses in distilled water.

The Philips EM 100B at KV 60 was used to observe sections. Micrographs were taken on 35mm Kodak Fine Grain Positive film (P426), and developed in Kodak developer D19.

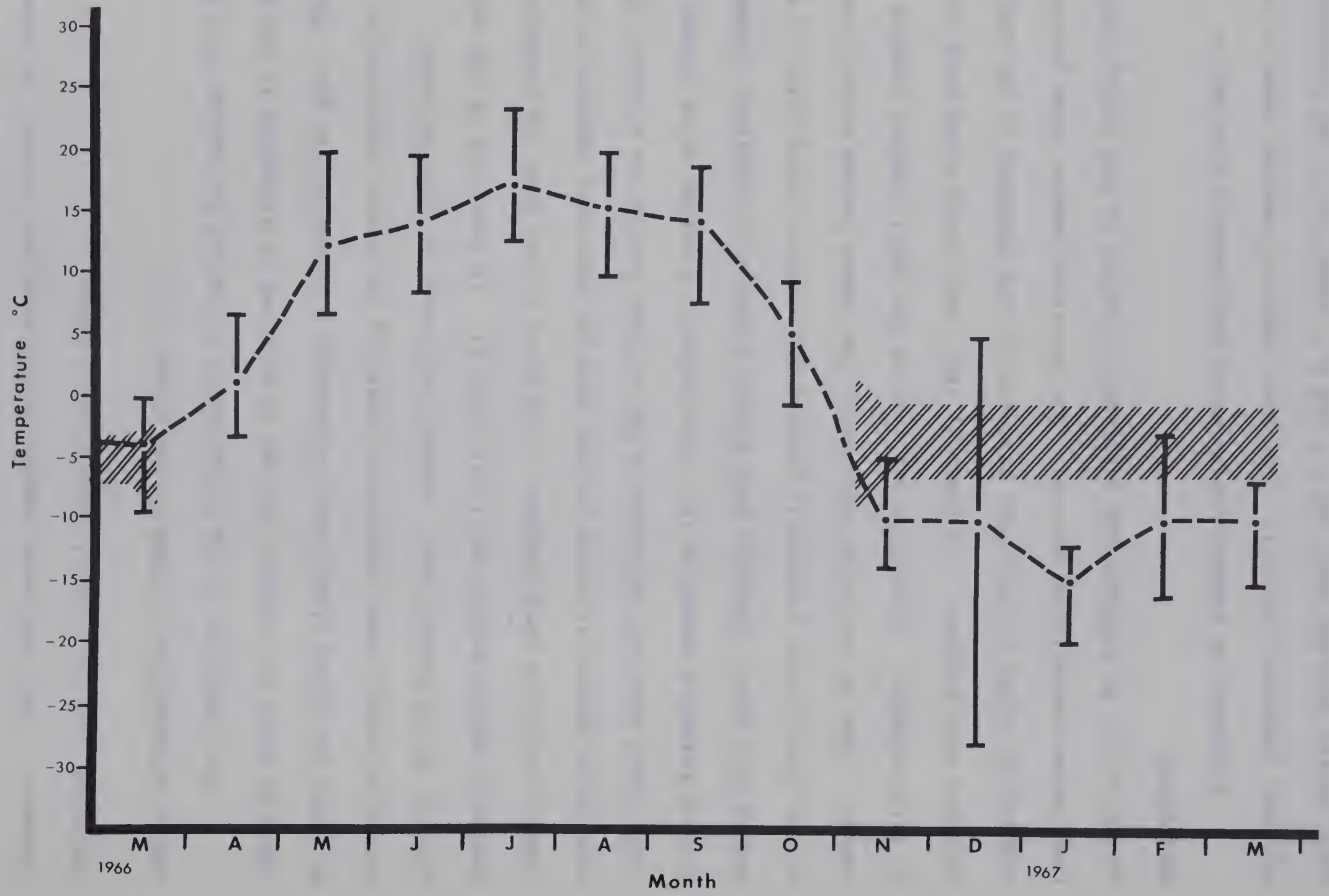
RESULTS

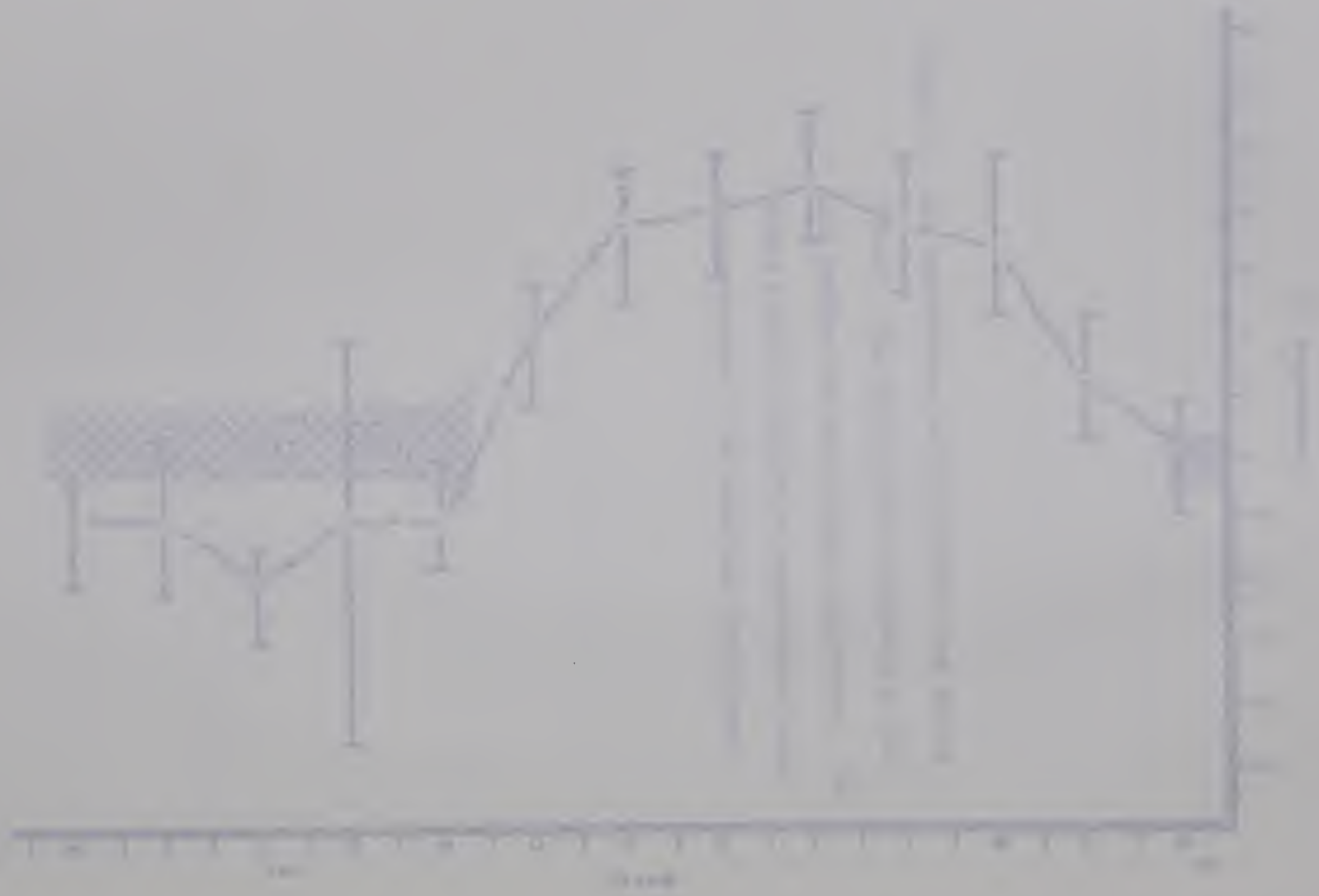
SEASONAL FLUCTUATIONS OF ENVIRONMENTAL TEMPERATURE

Mean monthly temperatures for the Edmonton area during 1966-67 are shown in Figure 1. The lowest mean minimum temperature was -27°C (December, 1966) and the highest mean maximum temperature was 23°C (July, 1966). Snow is present for at least five months of the year, from November to April. Snow was almost completely melted in the Edmonton area by the first week of April, 1966, and the mean air temperature at that time was 5.5°C. The mean monthly temperature increased steadily until July. High temperatures were maintained throughout the summer and began to decline after September. The most rapid drop in air temperature occurred in late October and early November just prior to the first snowfall. Snow cover began on 12 November and reached a depth of 25 cm by the end of that month.

Microhabitat temperatures taken throughout the study period provided some indication of temperatures to which voles were exposed under natural environmental conditions. During the period in which no snow was present, the microhabitat temperature of the meadow vole was 1 - 3°C lower than the air temperature taken several feet above ground. Snow, on the other hand, provides an insulative covering to the microhabitat. Pruitt (1957) states that a critical depth of 15cm of snow is necessary to stabilize subnivean temperatures at near freezing and permit burrowing by small mammals. The subnivean temperature ranged from -2°C to -8°C in vole habitat during the winter phase of this study (shaded portion on Fig. 1). It has been suggested

Figure 1. Air temperatures in the Edmonton area during 1966-67. The vertical bars terminate at the mean maximum and mean minimum temperature. The shaded portion indicates an approximate range of temperature in the subnivean environment.


Temperature °C
30
25
20
15
10
5
0
-5
-10
-15
-20
-25
-30
M
A
M
J
J
A
S
O
N
D
J
F
M
1966
1967
Month



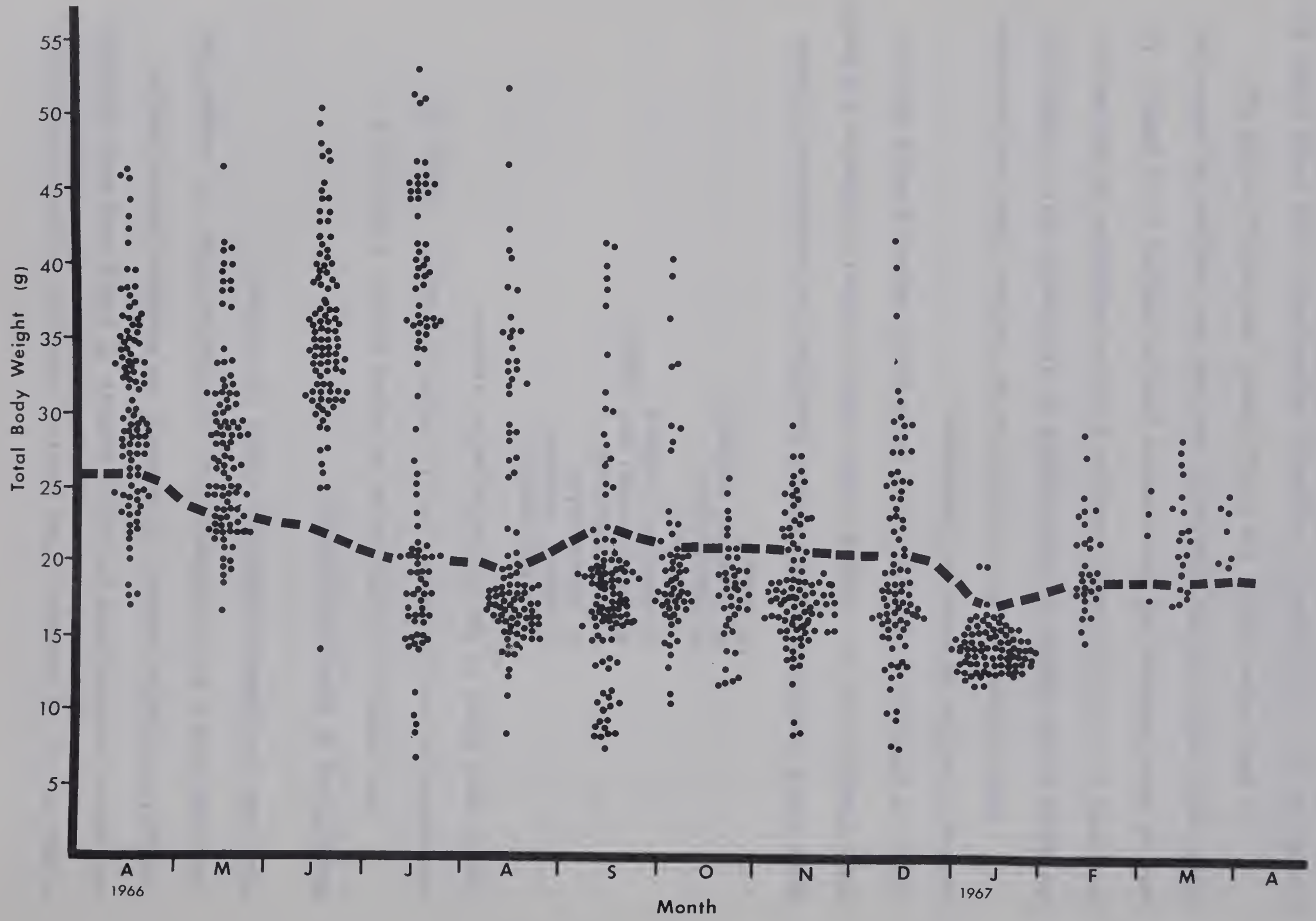
(Formozov, 1963) that such temperature fluctuations beneath the snow are dependent upon the thermal properties of snow.

ANNUAL REPRODUCTION PATTERN OF THE MEADOW VOLE

The condition of the reproductive system of the meadow vole was noted in order to estimate the age of mice and to determine if the mass of brown fat shows significant differences with respect to age. These data also supply some information regarding the annual reproductive pattern of the meadow vole. Animals surviving the 1965-66 winter gradually gained weight until July (Fig.2). In June, 45% of the mature females collected were pregnant. The first litter from the overwintering population appears to occur in late June and they were sampled in July. Young voles were not collected in May or June and may be a result of delayed breeding caused by the large numbers of meadow voles present during this time. However, more direct evidence is required. Members of the first litter presumably matured and produced young later in the summer. Some of the voles born during the summer became sexually mature in late November. Sixty-five per cent of the adult females sampled in December were pregnant. In January, 1967, only young voles were collected suggesting either a litter was produced in late December or the adults that became sexually mature died. The surviving meadow voles gradually gained weight by February and continued to mature as the spring conditions approached.

Pregnancy of females was observed continuously from May to December (Appendix Table II). The mean number of embryos found in the uteri during June and July was 6.5 and 6.1 respectively and decreased to

Figure 2. Variations in total body weight of the meadow vole. Each point represents one individual. The dotted line separates mature (above) and immature (below) voles.


Total Body Weight (g)
55
50
45
40
35
30
25
20
15
10
5
A
1966
M
J
J
A
S
O
N
D
J
1967
F
M
A
Month

a mean of 3.8 in December. The survival rate per litter could not be determined from the data but large numbers of young mice were collected during the breeding season. In October and November mature females contained from 2 to 12 placental scars (Appendix Table II), suggesting that some individuals produced at least two litters.

DESCRIPTION OF BROWN FAT DEPOSITS

The anatomical distribution of brown adipose tissue in *M. pennsylvanicus* was consistent in all animals examined. Brown fat depots were found in the following major regions:

1. interscapular
2. dorsal cervical
3. subscapular
4. suprascapular
5. suprasternal and jugular
6. carotid triangle
7. axillary
8. pericardial
9. internal thoracic
10. renal

These areas correspond to the depots described for *Peromyscus* by Rauch (MSc. Thesis, U of A). Similar areas were described by Cameron and Smith (1964) in young mice; however, they found minor deposits which are not present in either *Microtus* or *Peromyscus*.

Brown adipose tissue is deposited in discrete lobes and surrounds the major blood vessels in many regions of the meadow vole. Brown fat was found in all the animals examined with the exception of one mature male in late February and three mature females sampled on 31 March. In these cases brown fat was completely depleted and replaced by white fat.

In many voles during the fall, winter, and spring, white fat surrounded the interscapular, axillary, suprasternal, and renal brown fat

deposits (Fig. 3a). The white adipose tissue was separated from brown fat by a thin membrane and was easily removed. Very little white fat was present in the voles sampled during the summer, and the size of brown fat deposits was reduced (Fig. 3b). In many cases very little, if any, brown fat was found in the pericardial, internal thoracic and renal areas.

Seasonal variations in the color and characteristics of the tissue were observed. During the warmer months, especially June, the tissue in several mature individuals was soft and sticky in nature and dark red in color. In the colder months mature voles generally contained firmer deposits which were yellowish-red in color. Immature voles retained solid, yellowish-red tissue throughout the year, with some exceptions in the summer and late winter months.

Histological examination of brown fat cells from the meadow vole (Fig.4) showed a multilocular structure with rounded central nuclei similar to that observed by George and Eapen (1959) in the bat, and Cameron and Smith (1964) in the cold acclimated rat. Furthermore, an electron micrograph of brown fat taken from an adult female in the summer showed large numbers of mitochondria in close association with lipid droplets in the cytoplasm (Fig.5). Large numbers of mitochondria have been recorded in brown fat from newborn mice and rats (Napolitano and Fawcett, 1958), newborn rabbits (Hull, 1966), and bats (Hayward and Lyman, 1967). Structural changes in brown fat were not followed throughout the year.

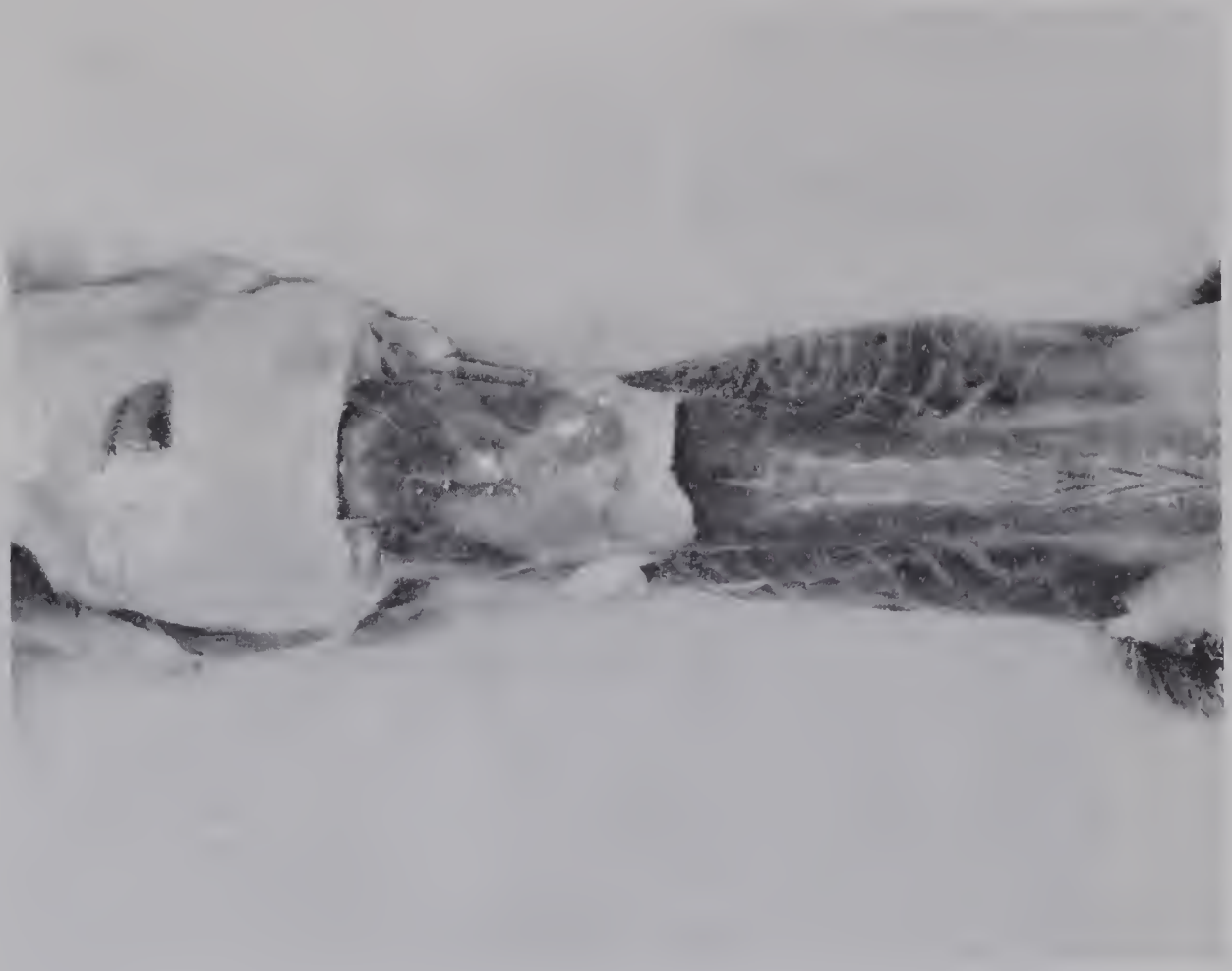
SEASONAL CHANGES IN THE MASS OF BROWN FAT AND TOTAL BODY WEIGHT

a. Immature Females and Males

Student's *t*-test was used to determine the significance of

Figure 3a. Interscapular brown fat in a meadow vole sampled during the winter. White adipose tissue surrounds the interscapular brown fat.

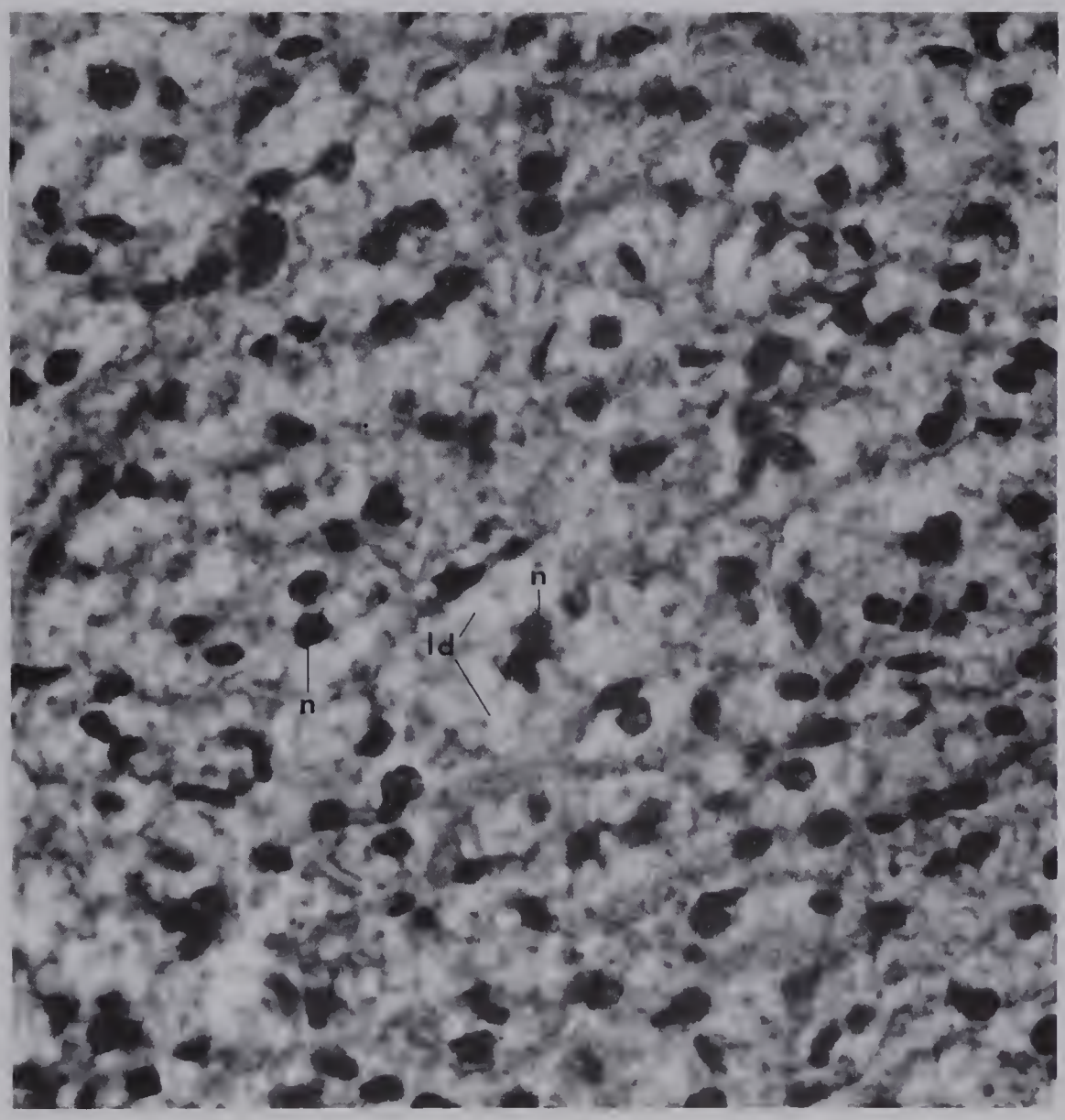
Figure 3b. Interscapular brown fat in a meadow vole sampled during the summer. White adipose tissue is absent.



(a)

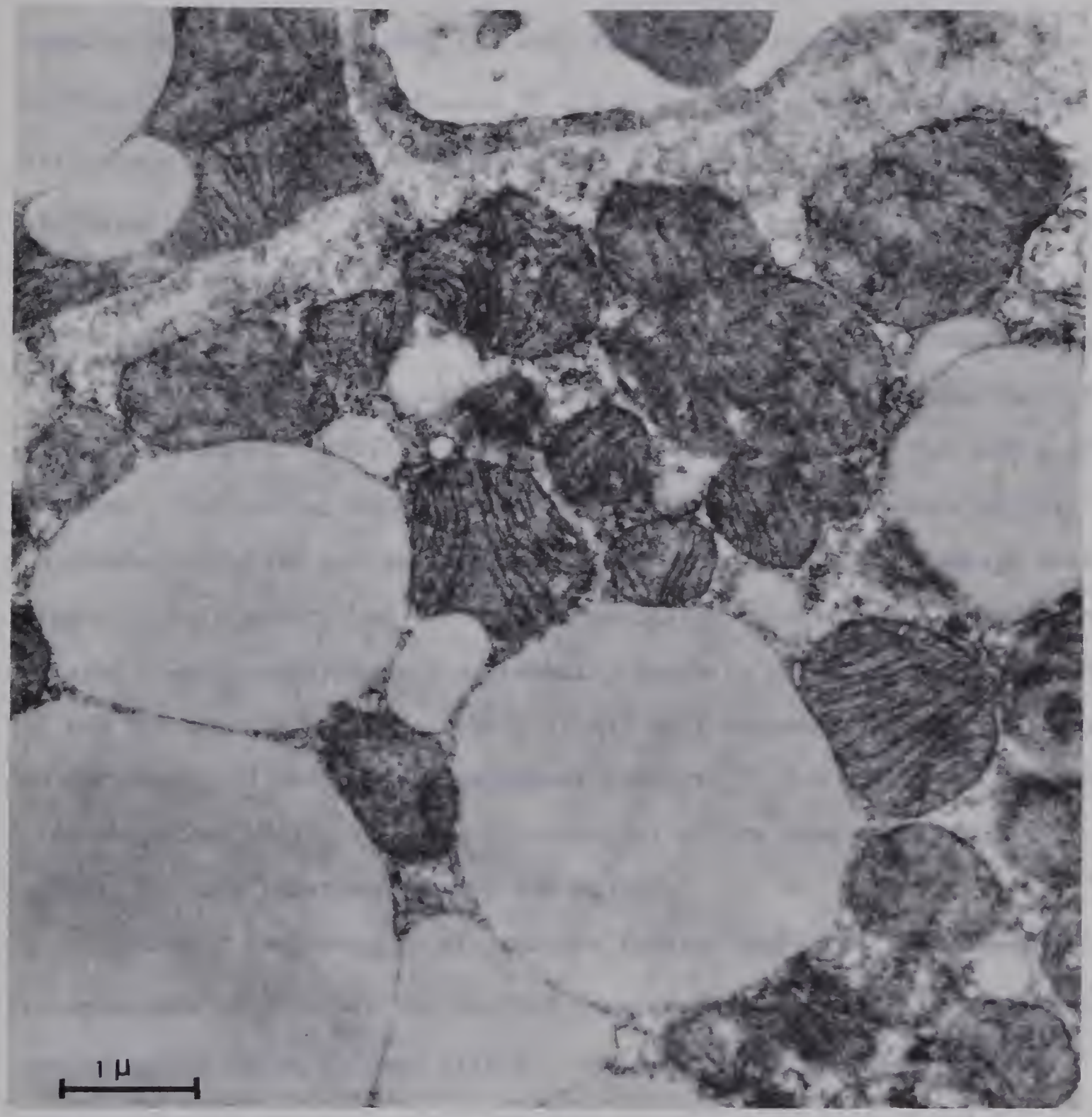
(b)

Figure 4. Photomicrograph of interscapular brown adipose tissue showing multilocular structure and central nuclei. Haemotoxylin and eosin stain. Magnification X620.
(n = nucleus, ld = lipid droplets)



100 μ

Figure 5. Electron micrograph of interscapular brown adipose tissue. Large mitochondria surround numerous droplets of fat. Magnification X18,000.


1 μ

differences between mean monthly amounts of brown adipose tissue (Appendix Table VI). The mean monthly mass of brown adipose tissue in immature females decreased from 300mg to 140mg from May to August (Fig.6). This 56% difference is highly significant ($P < 0.001$). There was no significant difference in the amount of brown fat between August and September but it increased significantly from September to January by a total of 121%. The decrease of brown fat from January to February is significant ($P < 0.01$), but the average quantity remained much higher than in the summer months (Fig.6).

The amount of brown adipose tissue in immature males decreased from 410mg to 170mg ($P < 0.01$) between April and July (Fig.7). This was a 58% decline. The loss of brown fat in immature males occurred more rapidly than in immature females and was minimal in July. No significant change occurred from July to October. As the summer conditions terminated, brown fat increased significantly over the preceding month in November and January by 47 and 28% respectively. A highly significant decrease ($P < 0.001$) occurred in February, followed by a significant increase ($P < 0.05$) in March. The pattern formed by the seasonal fluctuations in the mass of brown fat is similar in both immature females and males.

Monthly body weights of immature females and males were analyzed to determine if fluctuations in this parameter corresponded to changes in the weight of brown adipose tissue. The mean monthly body weight of immature female and male voles are also plotted on Figures 6 and 7. Mean body weight of immature females and males declined significantly between May and July and increased significantly between January and February (Appendix Table VIII). The decrease in July was probably a result of new individuals born in June. The increase from January to February may be caused by growth of voles born in late December. The decline in average body weight of immature males between October and

Figure 6. Variations in the mass of brown adipose tissue (solid line) and total body weight (broken line) of immature female voles. The vertical bars represent one standard deviation of the mean and the number above the bars represents the number of voles examined.

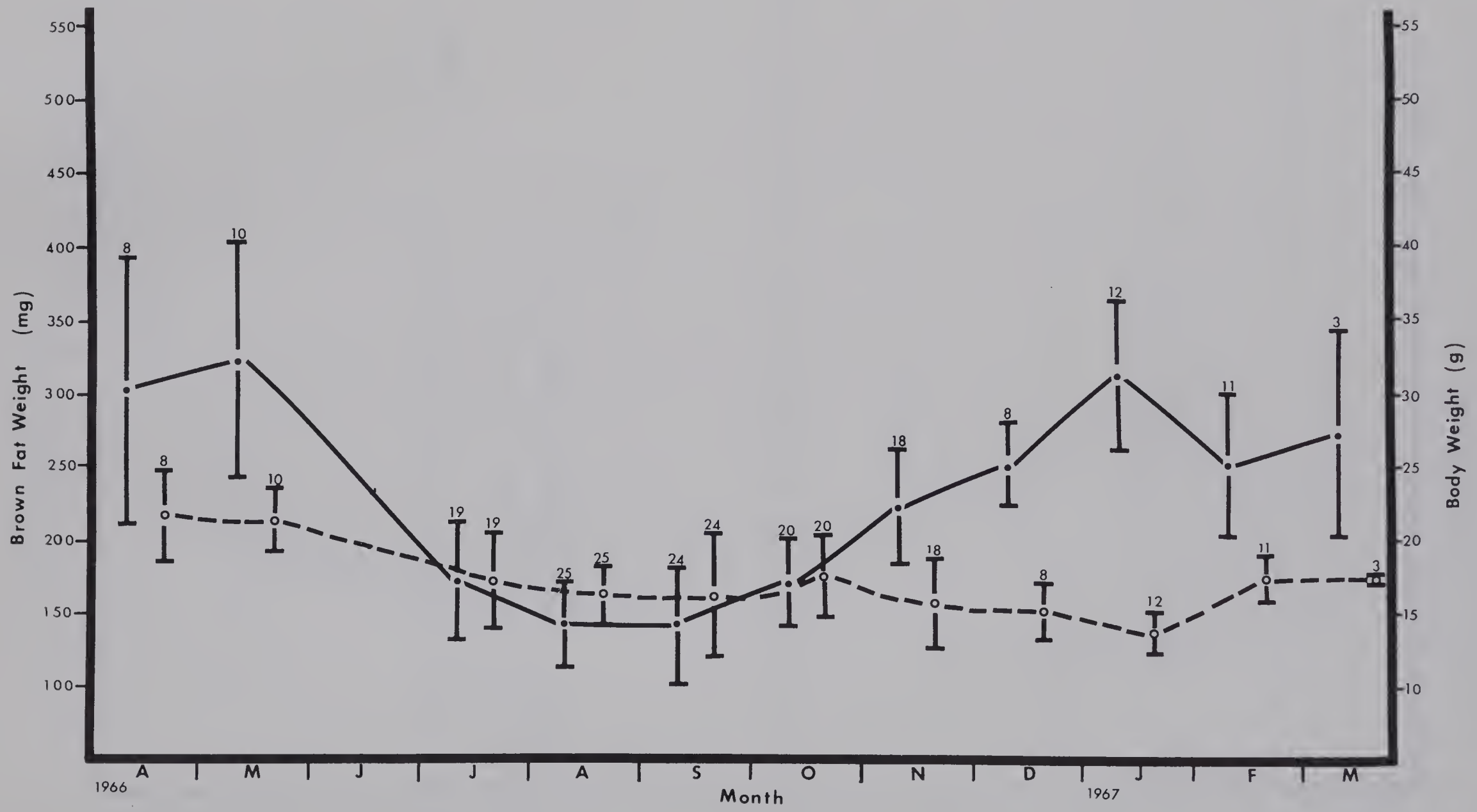
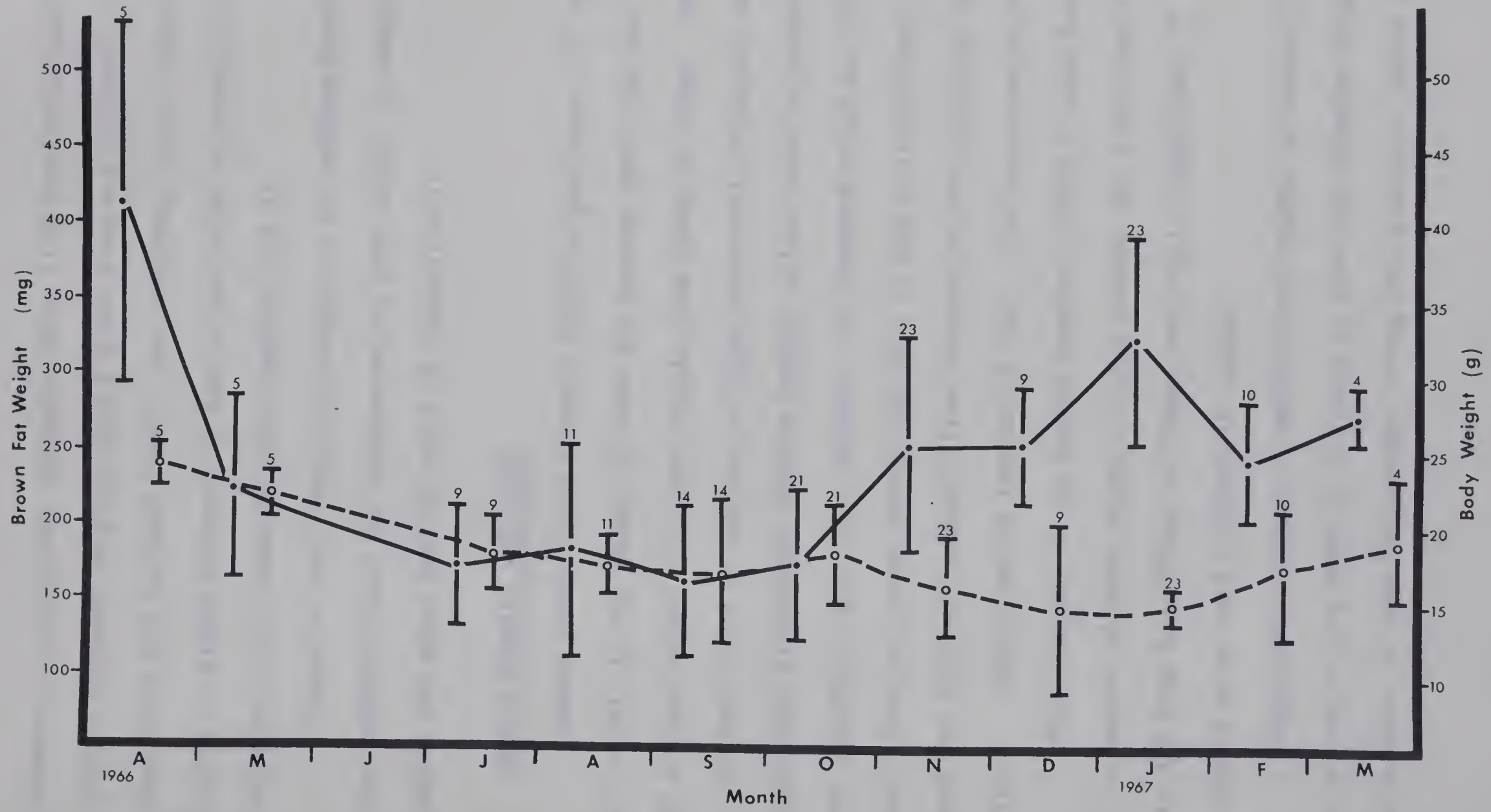

Brown Fat Weight (mg)
Body Weight (g)
Month
1966
1967
A
M
J
J
A
S
O
N
D
J
F
M
550
500
450
400
350
300
250
200
150
100
55
50
45
40
35
30
25
20
15
10
8
10
19
19
25
25
24
24
20
20
18
8
12
11
3
8
10
18
18
8
12
11
3

Figure 7. Variations in the mass of brown adipose tissue (solid) line) and total body weight (broken line) of immature male voles. Graphic presentation is the same as in Figure 6.


Brown Fat Weight (mg)
500
450
400
350
300
250
200
150
100
Body Weight (g)
50
45
40
35
30
25
20
15
10
A
M
J
J
A
S
O
N
D
J
F
M
1966
1967
Month

November is significant (Appendix Table VII) when three individuals caught 3 November weighing only 8.0 and 8.6g are included. If these three voles are excluded (n=41), the difference is not significant. However, a slight decrease in body weight prior to snowfall is indicated in all immature voles sampled (Fig.2).

Figures 6 and 7 show that changes in the average amount of brown adipose tissue are independent of body weight in immature females and males (r=0.47 and 0.34 respectively).

b. Mature Females and Males

Mature female voles showed a highly significant, 57% decrease ($P < 0.001$) in the amount of brown fat between April and June (Fig.8). The average weight of tissue dropped from 420mg to 180mg. Because of the small size of samples and high individual variation, no significant differences between monthly values could be detected after June (Appendix Table VI). However, the December value was 250% greater than values for summer months and this is highly significant ($P < 0.001$). Brown fat declined gradually from December to the following April (Fig.8). Several mature females in April, 1967 contained no brown fat.

Mature males, as the mature females, showed a rapid decrease in the amount of brown adipose tissue between April and June (Fig.9). This 66% drop from 470mg to 160mg was highly significant ($P < 0.01$). No mature males were obtained in January.

Significant changes in monthly body weights of mature females are related to the onset of breeding in June and December (Fig.8). The increase in mean body weight during this period is mainly a result

Figure 8. Variations in the mass of brown adipose tissue (solid line) and total body weight (broken line) of mature female voles. Graphic presentation is the same as in Figure 6.

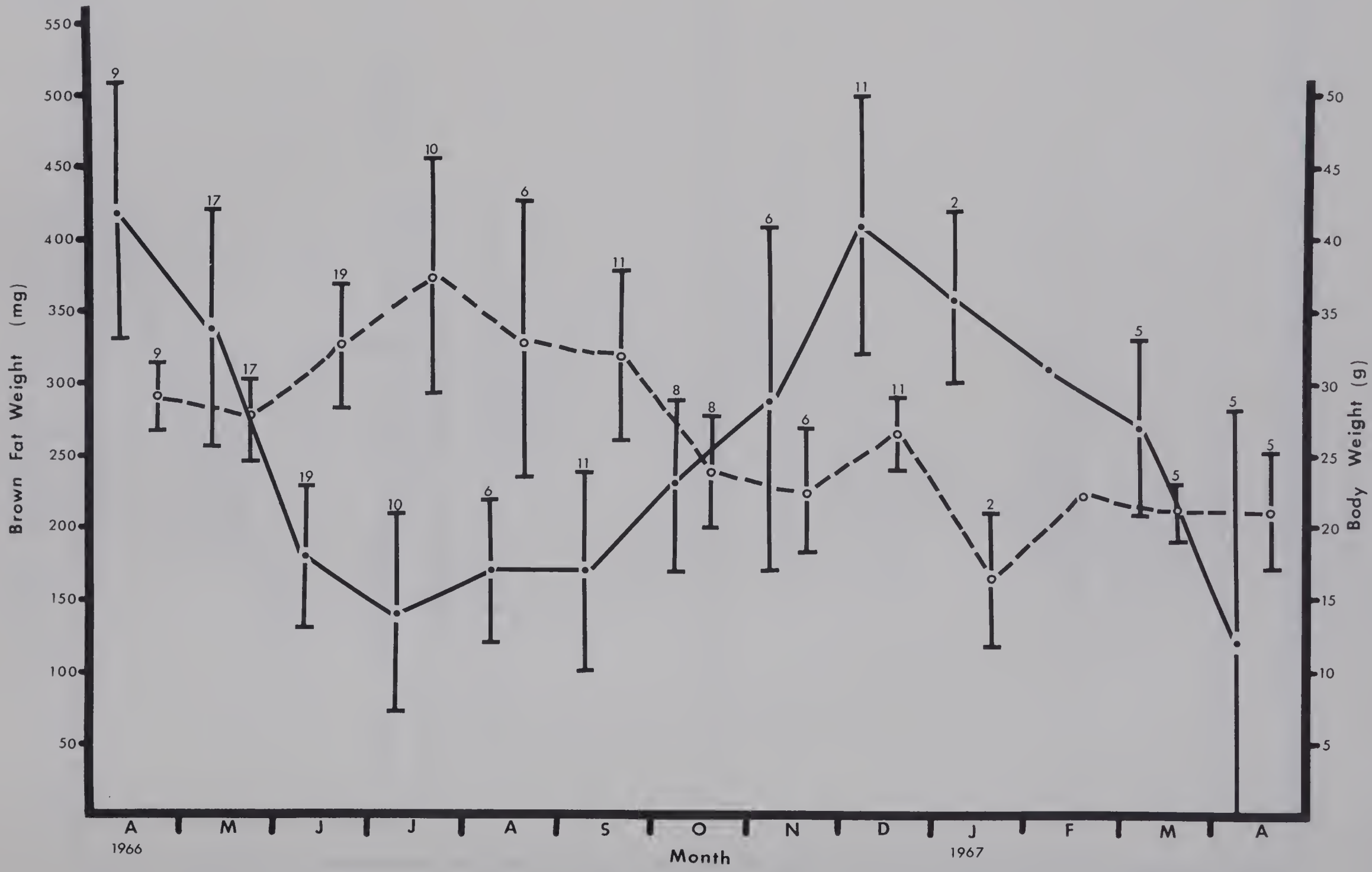
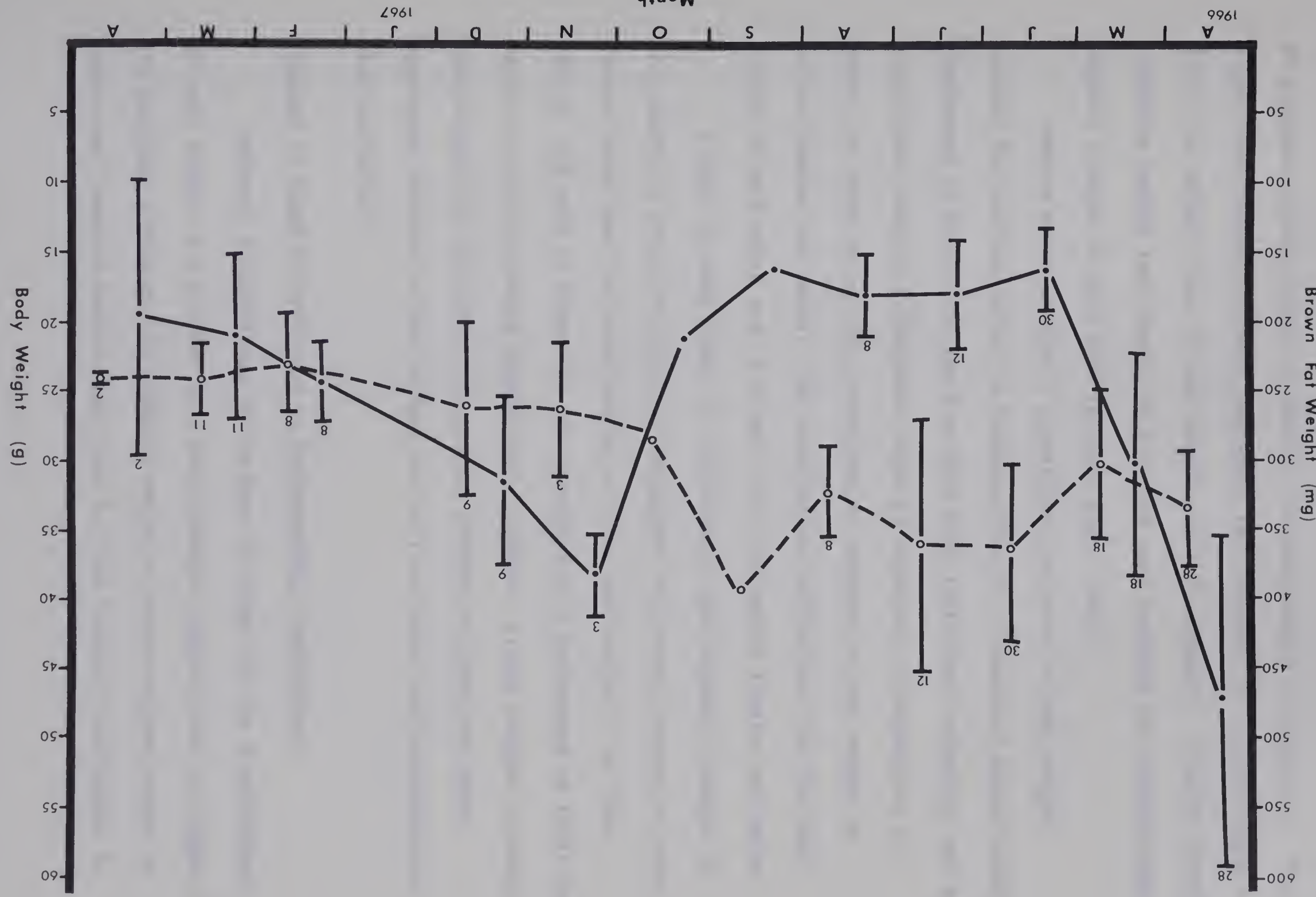

Brown Fat Weight (mg)
Body Weight (g)
550
500
450
400
350
300
250
200
150
100
50
50
45
40
35
30
25
20
15
10
5
A
M
J
J
A
S
O
N
D
J
F
M
A
1966
1967
Month

Figure 9. Variations in the mass of brown adipose tissue (solid line) and total body weights (broken line) of mature male voles. Graphic presentation is the same as in Figure 6.


Body Weight (g)
Brown Fat Weight (mg)
Month
A
M
J
J
A
S
O
N
D
J
F
M
A
1966
1967
5
10
15
20
25
30
35
40
45
50
55
60
50
100
150
200
250
300
350
400
450
500
550
600

of pregnancy (body weight of females included weight of embryos). The significant decrease between September and November ($P < 0.005$) might be due to an overall loss of body weight prior to snowfall. Similar findings regarding weight loss during this period were recorded for *Clethrionomys gapperi* studied in this area (Elliot, pers. comm.).

Mature males exhibit a significant increase in body weight between May and June prior to breeding (Fig.9). Remaining sample numbers (September to March) are small or have high individual variation, and no significant monthly differences could be detected. Fluctuations in amount of brown fat do not correspond to changes in body weight of mature females and males. The correlation coefficients for the mean values of each month are -0.04 and -0.02 for mature females and males.

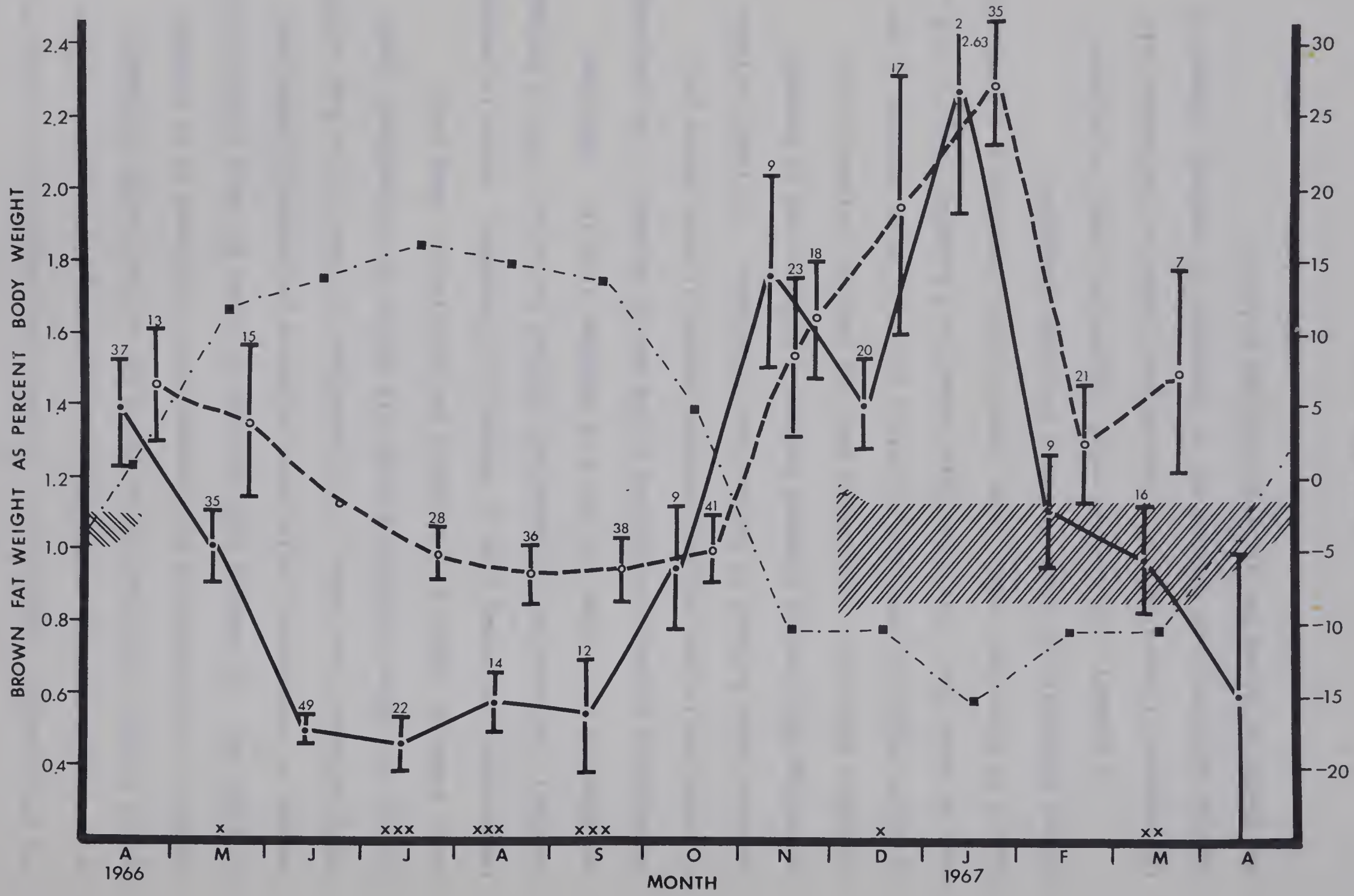
A point to make clear at this time is that dramatic changes in the amount of brown fat occurred throughout the season because of some factor other than the relationship to total body weight. For this reason, the mass of brown fat was expressed as a percentage of total body weight for the following comparisons. However, slight changes in monthly body weights do influence the seasonal pattern of brown fat when expressed relative to body weight and will be taken into consideration when warranted.

CHANGES IN BROWN FAT RELATIVE TO ENVIRONMENTAL TEMPERATURE

Seasonal fluctuations in the mass of brown fat (as a percentage of body weight) are plotted with environmental temperatures in Figure 10. The seasonal trends of the relative weight of brown adipose tissue in mature and immature meadow voles show a close inverse relationship to

Figure 10. Variations in the percentages of brown fat relative to body weight in mature (solid line) and immature (broken line) voles. The dash-dot line indicates the mean air temperature of the Edmonton area during 1966-67, and the shaded portion indicates an approximate range of temperature in the subnivean environment. The vertical bars represent two standard errors of the mean and the numbers above the bars represent the number of voles sampled. Significant differences between immature and mature voles in any one month are indicated above the month on the abscissa of the graph as follows:

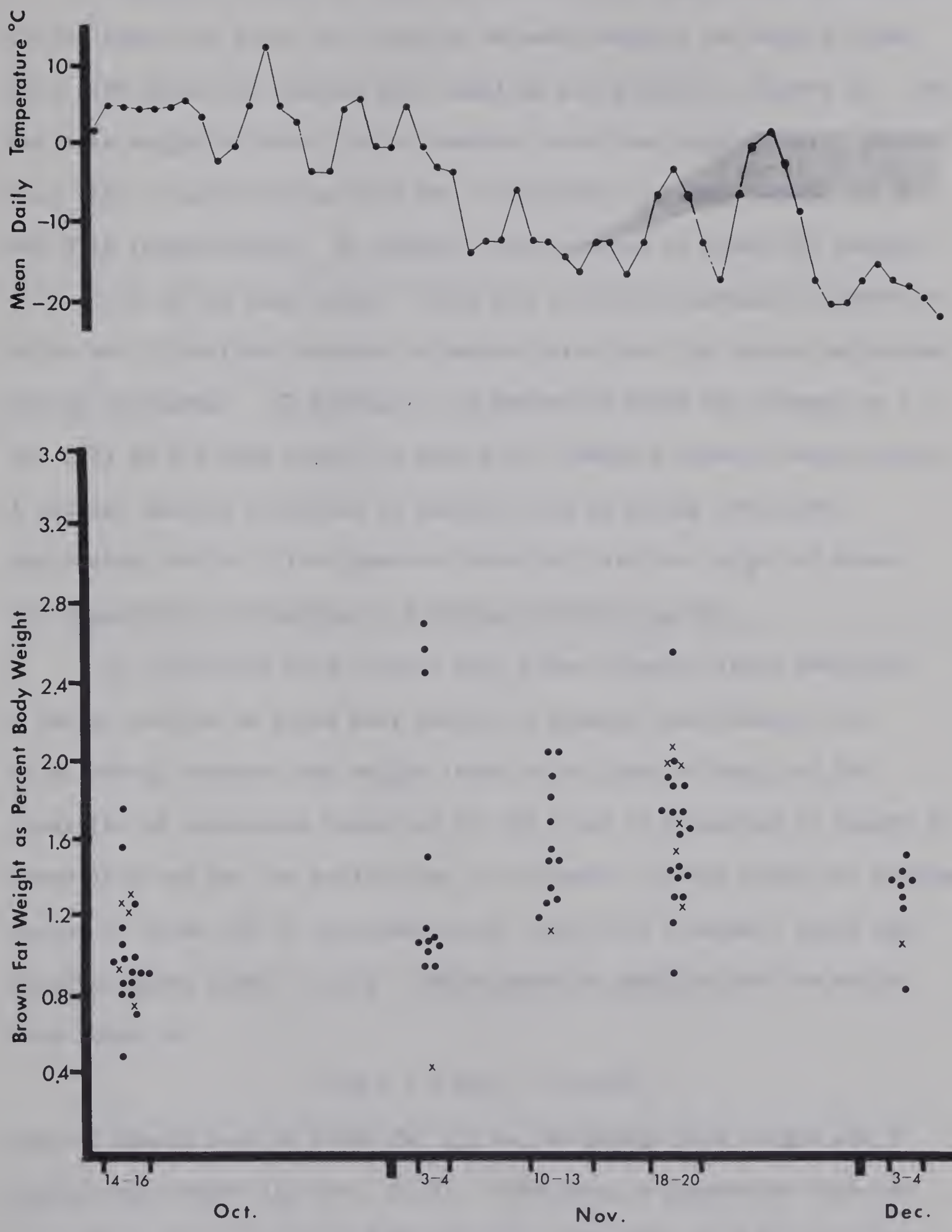
x significant at the 1% level
xx significant at the 0.5% level
xxx significant at the 0.1% level.


BROWN FAT WEIGHT AS PERCENT BODY WEIGHT
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
TEMPERATURE °C
30
25
20
15
10
5
0
−5
−10
−15
−20
37
13
15
35
28
36
38
9
41
9
23
18
20
17
2
2.63
35
9
21
16
7
49
22
14
12
x
xxx
xxx
xxx
x
xx
A
1966
M
J
J
A
S
O
N
D
J
F
M
A
1967
MONTH

the environmental temperature. The subnivean temperature is indicated by the shaded portion on Figure 10. As the temperature rises in late spring (1966), the proportion of brown fat to body weight decreases. Similarly, the decreasing fall temperature is followed by an increase in brown fat. To examine this increase in brown fat more closely with respect to temperature, voles were collected periodically before and after permanent snow cover. The percentage of brown fat to body weight for all the voles collected during this period was determined. Each point shown on Figure 11 represents one individual. Mean daily temperatures are shown from 14 October to 4 December. Prior to permanent snowfall, the mean daily temperature dropped as low as -14°C (5 November) and remained well below 0°C until 24 November (Fig.11). Subnivean temperatures probably stabilized by the end of November. The percentage of brown fat to body weight increased twofold in both mature and immature voles between 14 October and 20 November. A slight increase in average body weight occurred between 3 November and 20 November (Appendix Table VIII) indicating that the twofold increase in the relative weight of brown fat is solely due to a large increase in the mass of brown fat. The three high values on 3 November (Fig.11) are a result of three very young voles sampled which contained a significantly higher proportion of brown fat than the older juveniles.

In summary, the environmental temperature seems to influence the seasonal fluctuations of brown adipose tissue in the meadow vole. Further analyses were carried out to determine if seasonal changes in the amount of brown fat differed with age and sex.

Figure 11. Variations of the relative weight of brown fat in immature (●) and mature (x) voles. Each mark represents one individual. Mean daily temperatures are connected to show trend and the shaded portion indicates an approximate temperature range in the subnivean environment.


Mean Daily Temperature °C
10
0
−10
−20
Brown Fat Weight as Percent Body Weight
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
14 – 16
3–4
10 – 13
18 – 20
3–4
Oct.
Nov.
Dec.

a. Comparison of Brown Fat in Immature and Mature Mice

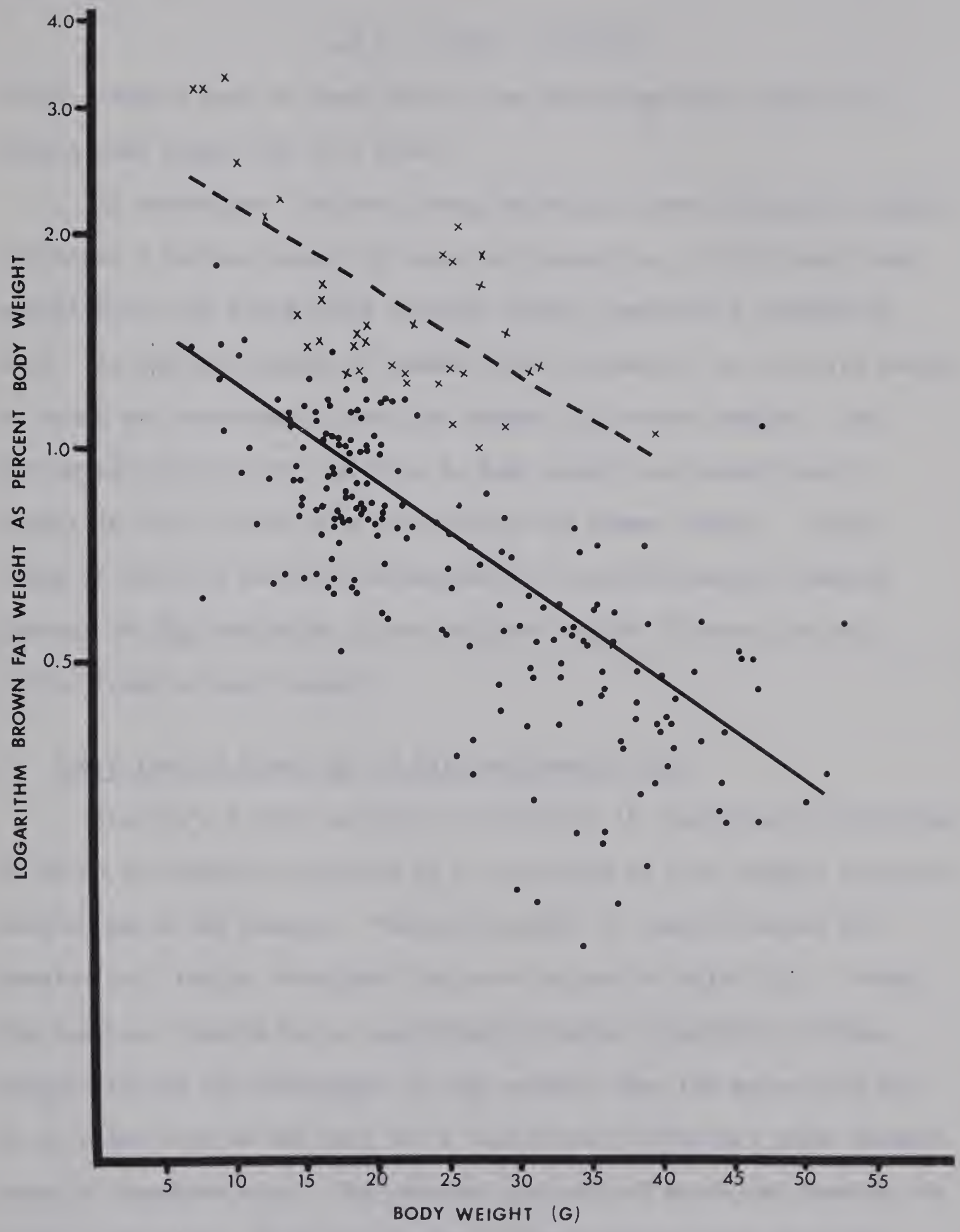
Student's *t*-test was used to determine if significant differences in the amount of brown fat occurred between immature and mature voles. Data from males and females were combined and plotted in Figure 10. The relative weight of brown fat in immature voles was significantly greater than that in mature voles from May to September (average amount is 1% and 0.5% respectively). By January, the increase in brown fat reached 2.0 - 2.5% of the body weight. This was a twofold increase in immature voles and a fourfold increase in mature voles over the levels maintained during the summer. In February, the amount of brown fat dropped to 1.1 and 1.3% of the body weight in mature and immature animals respectively. A gradual decline continued in mature voles as spring conditions approached, while in the immature voles the relative weight of brown fat appears not to decline to a similar extent (Fig.10).

To illustrate more clearly that brown adipose tissue comprises a larger portion of total body weight in younger individuals, the relationship between body weight (used as an index of age) and the logarithm of percentage brown fat of 200 voles is presented in Figure 12. These data are for the period June to September, during which the average amount of brown fat of individuals was relatively constant, while body weights ranged from 7 to 52g. The regression equation for the solid line shown is:

$$\log Y = 0.2384 - 0.0142X$$

where Y equals mass of brown fat (g) as percentage body weight and X equals body weight (g) ($r = -0.77$). Similarly, a regression line was drawn from the data obtained for December (Fig.12). The body weights

Figure 12. Relationship between body weight and logarithm of brown fat weight as per cent body weight in voles sampled in June, July, August, and September (●) and December (x). Each mark represents one individual vole.


LOGARITHM BROWN FAT WEIGHT AS PERCENT BODY WEIGHT
4.0
3.0
2.0
1.0
0.5
5
10
15
20
25
30
35
40
45
50
55
BODY WEIGHT (G)

ranged from 8 to 42g. The regression equation for the broken line shown is:

$$\log Y = 0.4686 - 0.0122X$$

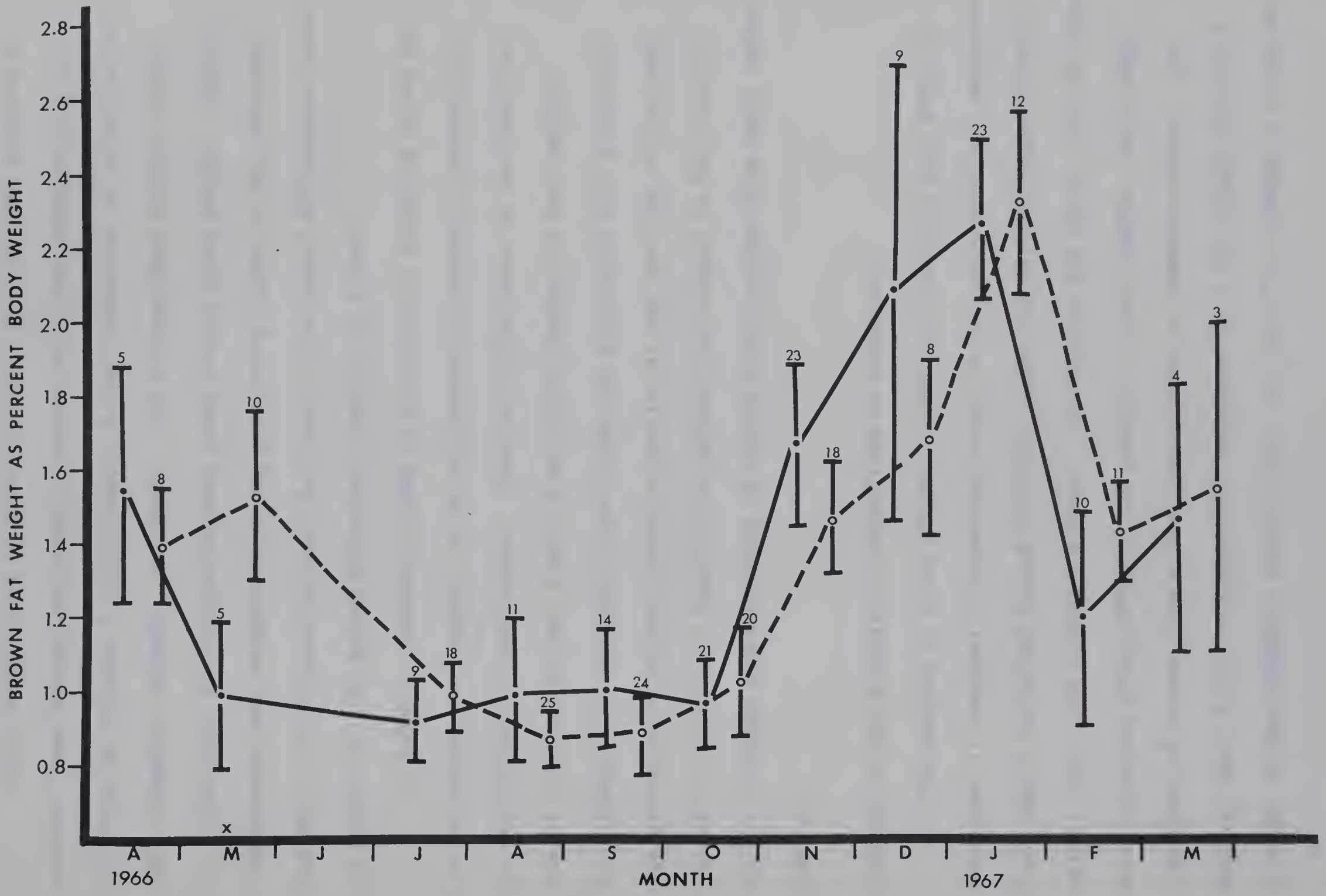
where Y equals mass of brown fat (g) as percentage body weight and X equals body weight (g) ($r = 0.64$).

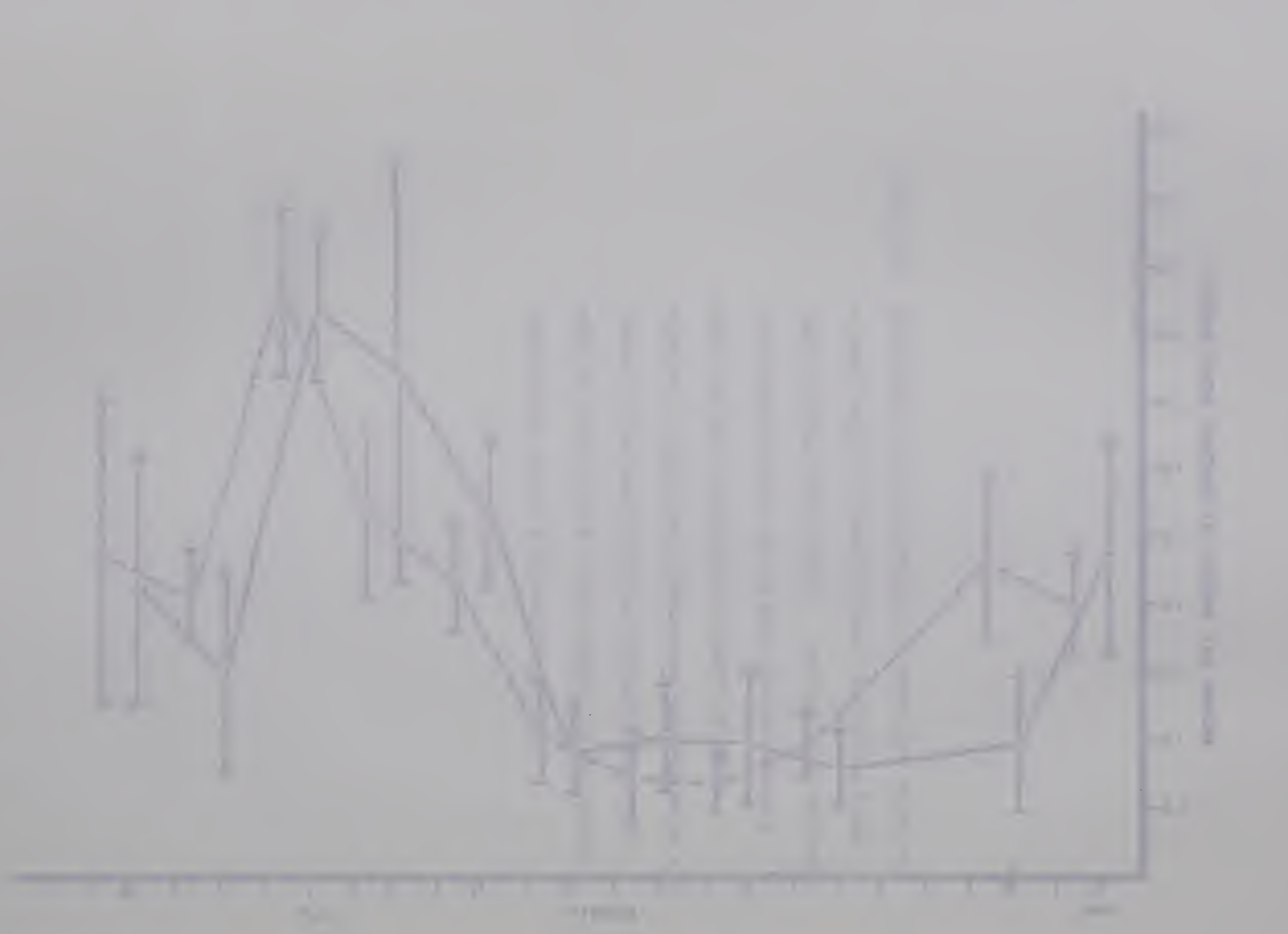
In comparison, the very young individuals born during the summer contained a maximum amount of brown fat comprising 1.9% of their body weight while the young voles in early winter contained a maximum of 3.3%. As the body weight of meadow voles increased, the relative weight of brown fat decreased in both the summer and winter samples. The percentage of brown fat relative to body weight was significantly higher in early winter than that during the summer months. Similar lines of best fit were not determined for the other monthly samples because of high variation in the relative weight of brown fat and narrow range of body weights.

b. Comparison of Brown Fat in Male and Female Voles

Student's *t*-test was used to determine if significant differences in brown fat weight (expressed as a percentage of body weight) occurred between males and females. The body weights of immature males and females are similar throughout the year (Appendix Table III). In May, the immature females had a significantly higher proportion of brown adipose tissue (as percentage of body weight) than the males (Fig.13). At no other time of the year did a significant difference exist between sexes of immature mice. The seasonal patterns of males and females are similar (Fig.13). The males tended to reduce their brown fat more rapidly in the spring and build it up more rapidly in the fall.

Figure 13. Variations in the percentage of brown fat relative to body weight in immature male (solid line) and female (broken line) voles. Vertical bars represent two standard errors of the mean and the numbers above the bars represent the number of voles examined. Significant differences between males and females in any one month are indicated above the month on the abscissa of the graph. (x represents significance at the 0.5% level.)


BROWN FAT WEIGHT AS PERCENT BODY WEIGHT
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
A
M
J
J
A
S
O
N
D
J
F
M
1966
1967
MONTH
x



Sexual differences in relative weight of brown fat occurred at several times during this study in mature males and females (Fig.14). The females retained a significantly higher percentage of brown fat in May, November, December, and March. The average body weights showed no significant differences between sexes during these months. However, the average body weights of mature males were higher in May, November, and March, which would account for some of the sexual differences shown in Figure 14. No mature males were sampled in January.

In summary, seasonal trends in the relative weight of brown fat in the meadow vole appear to be influenced by seasonal fluctuations in environmental temperatures. However, differences in the relative weight of brown fat may result from slight changes in body weight. Furthermore, the seasonal trends shown are associated with different age groups of voles that comprise samples at any one time of the year. Therefore, caution is required in emphasizing changes in the relative weight of brown adipose tissue of meadow voles sampled from their natural habitat.

CHANGES IN GROSS CHEMICAL COMPOSITION OF BROWN FAT

Ten samples of brown adipose tissue were analyzed each month to determine if seasonal fluctuations occur in the gross chemical composition. Each sample contained tissue from five voles and was analyzed for total water, lipid, and nitrogen content. In February and March, only 60 voles were collected which provided six samples for each month. Each sample consisted of tissue from either mature voles or immature voles. The monthly mean of each component are expressed on a wet weight basis and plotted to show seasonal trends (Figs. 15, 16, 17). Student's *t*-test was

Figure 14. Variations in the percentage of brown fat relative to body weight in mature male (solid line) and female (broken line) voles. Vertical bars represent two standard errors of the mean and the numbers above the bars represent the number of voles examined. Significant differences between males and females in any one month are indicated above the month on the abscissa of the graph as follows:

x = significant at the 5% level
xx = significant at the 2.5% level
xxx = significant at the 1% level.

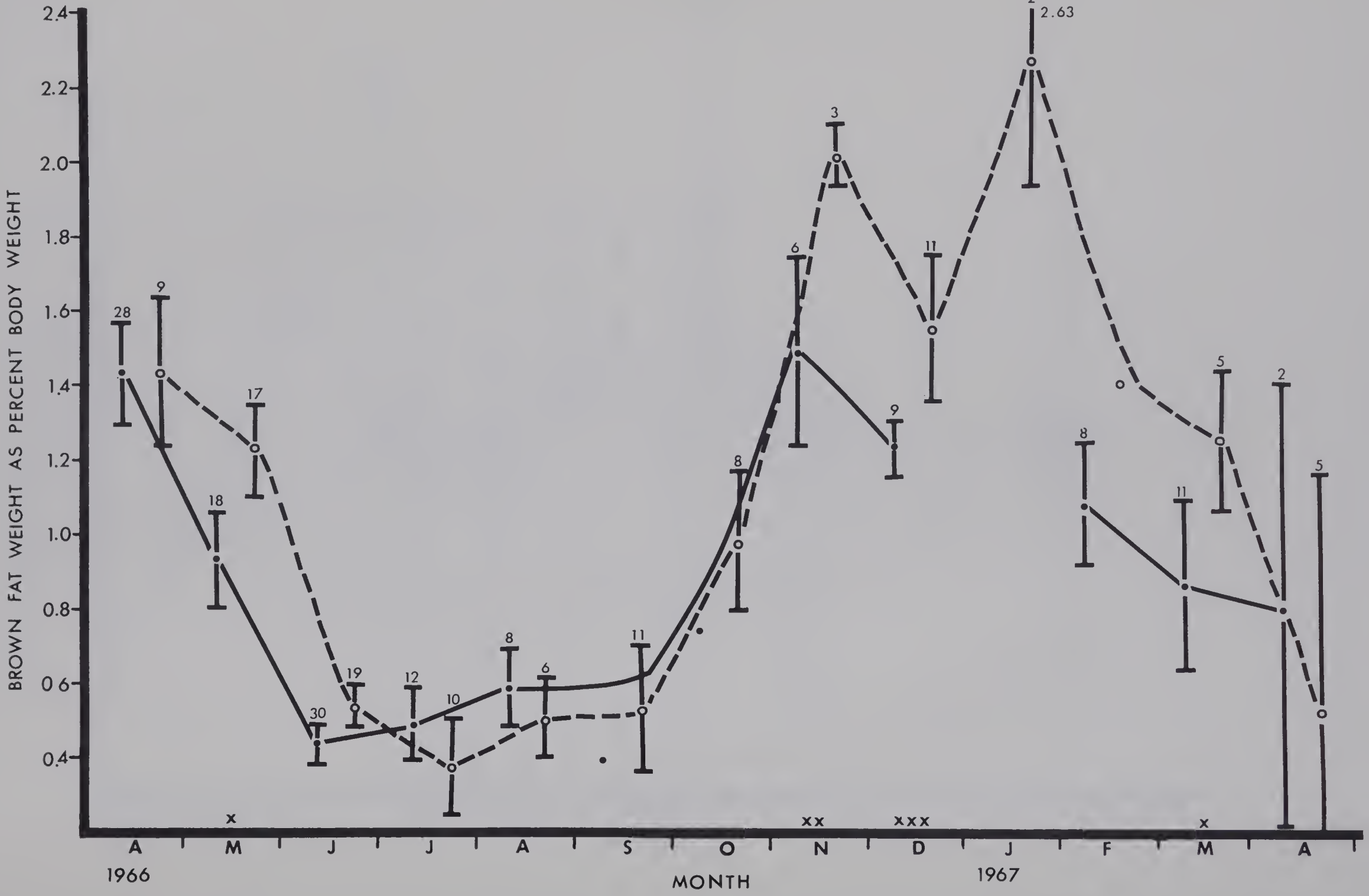

BROWN FAT WEIGHT AS PERCENT BODY WEIGHT
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
28
9
17
18
30
19
12
10
8
6
11
3
6
9
8
2
2.63
8
11
5
2
5
x
xx
xxx
x
A
M
J
J
A
S
O
N
D
J
F
M
A
1966
MONTH
1967

Figure 15. Variations in the lipid content of brown fat in mature (solid line) and immature (broken line) voles. The vertical bars represent one standard deviation of the mean and the numbers above the bars represent the number of samples. Significant differences between immature and mature voles in any one month are indicated above the month on the abscissa of the graph as follows:

x significant at the 2% level
xx significant at the 0.5% level
xxx significant at the 0.1% level.

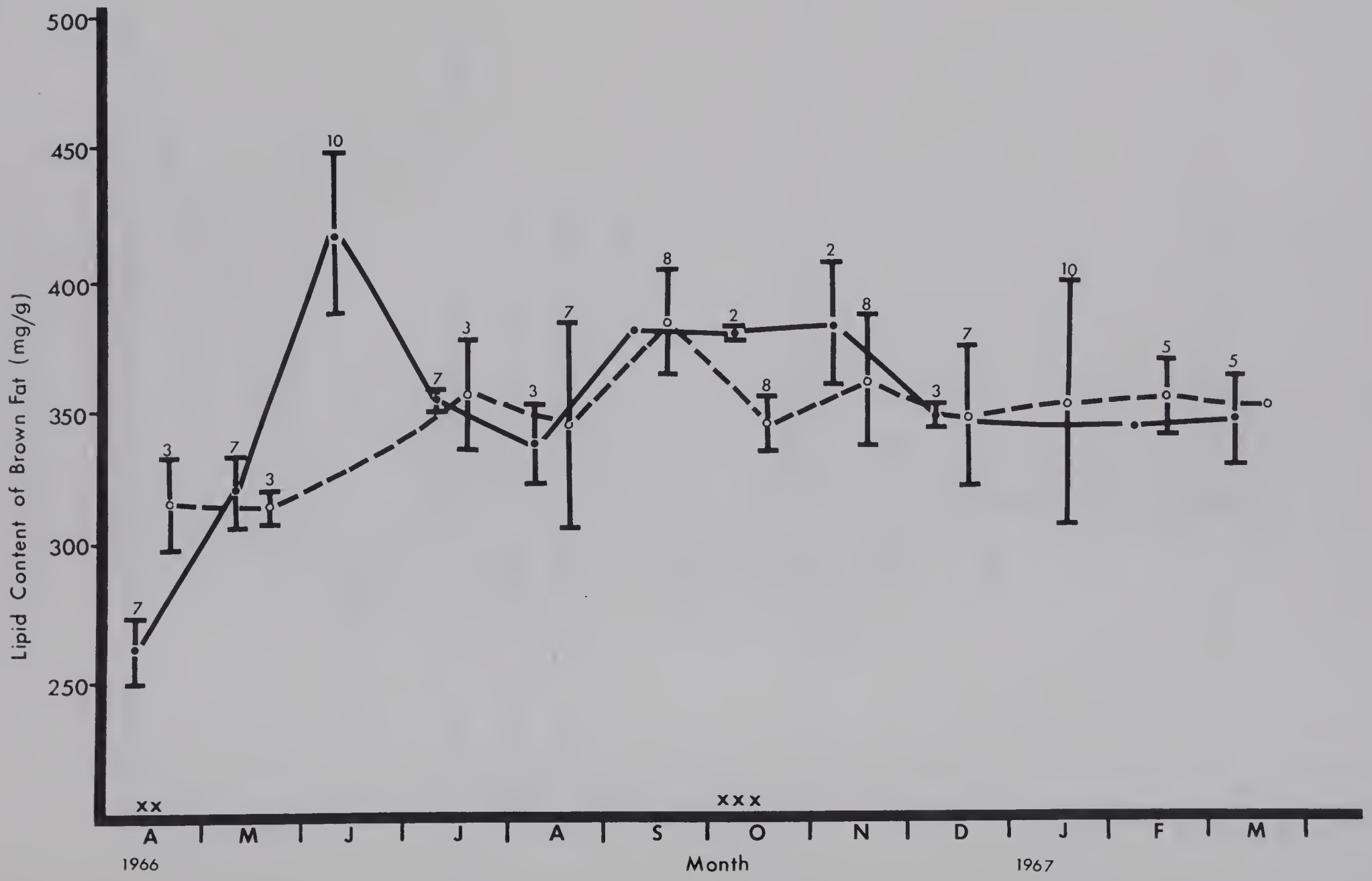

Lipid Content of Brown Fat (mg/g)
500
450
400
350
300
250
xx
xxx
A
M
J
J
A
S
O
N
D
J
F
M
1966
1967
Month

Figure 16. Variations in the water content of brown fat in mature (solid line) and immature (broken line) voles. Graphic presentation is the same as in Figure 15.

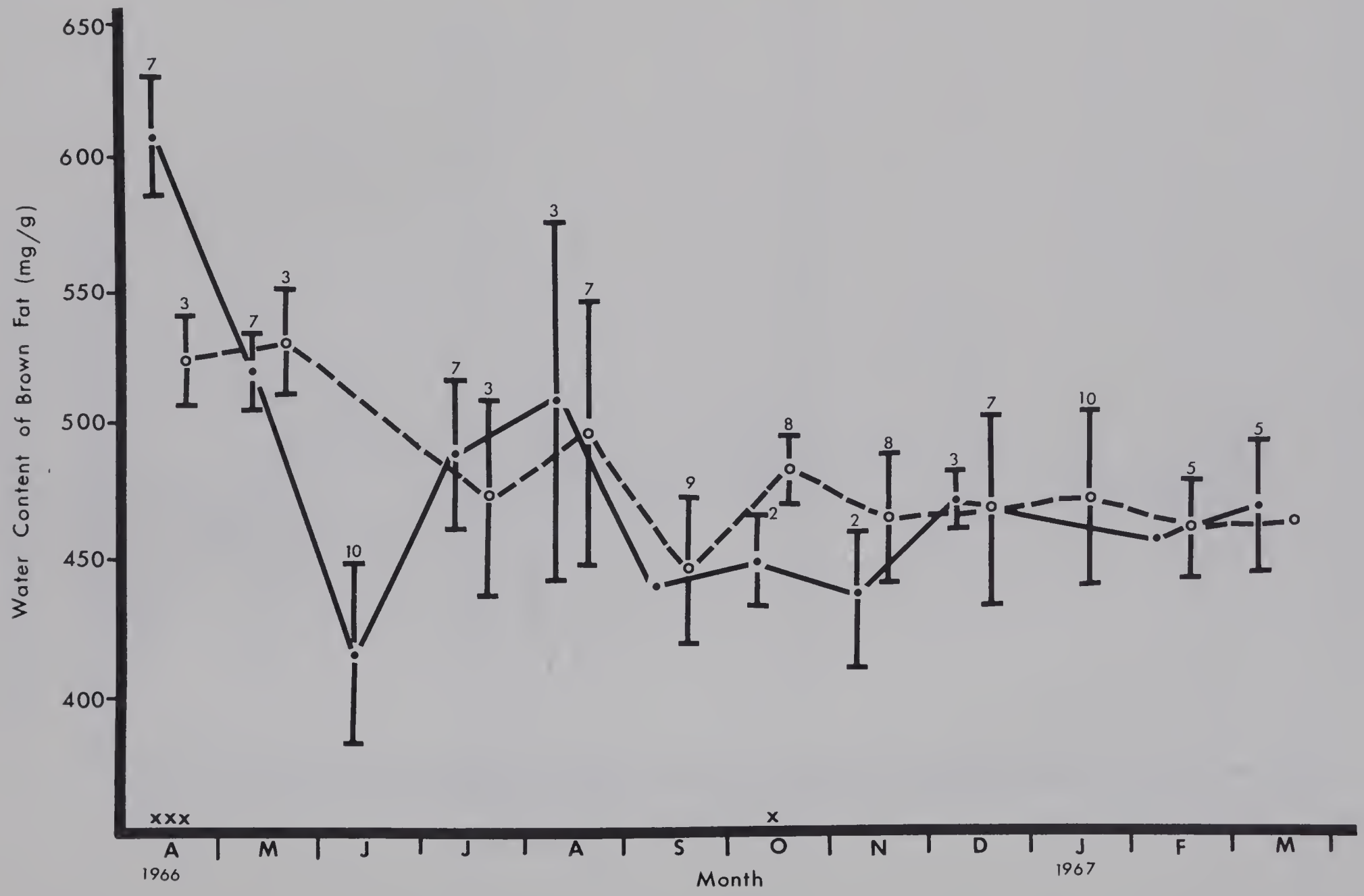
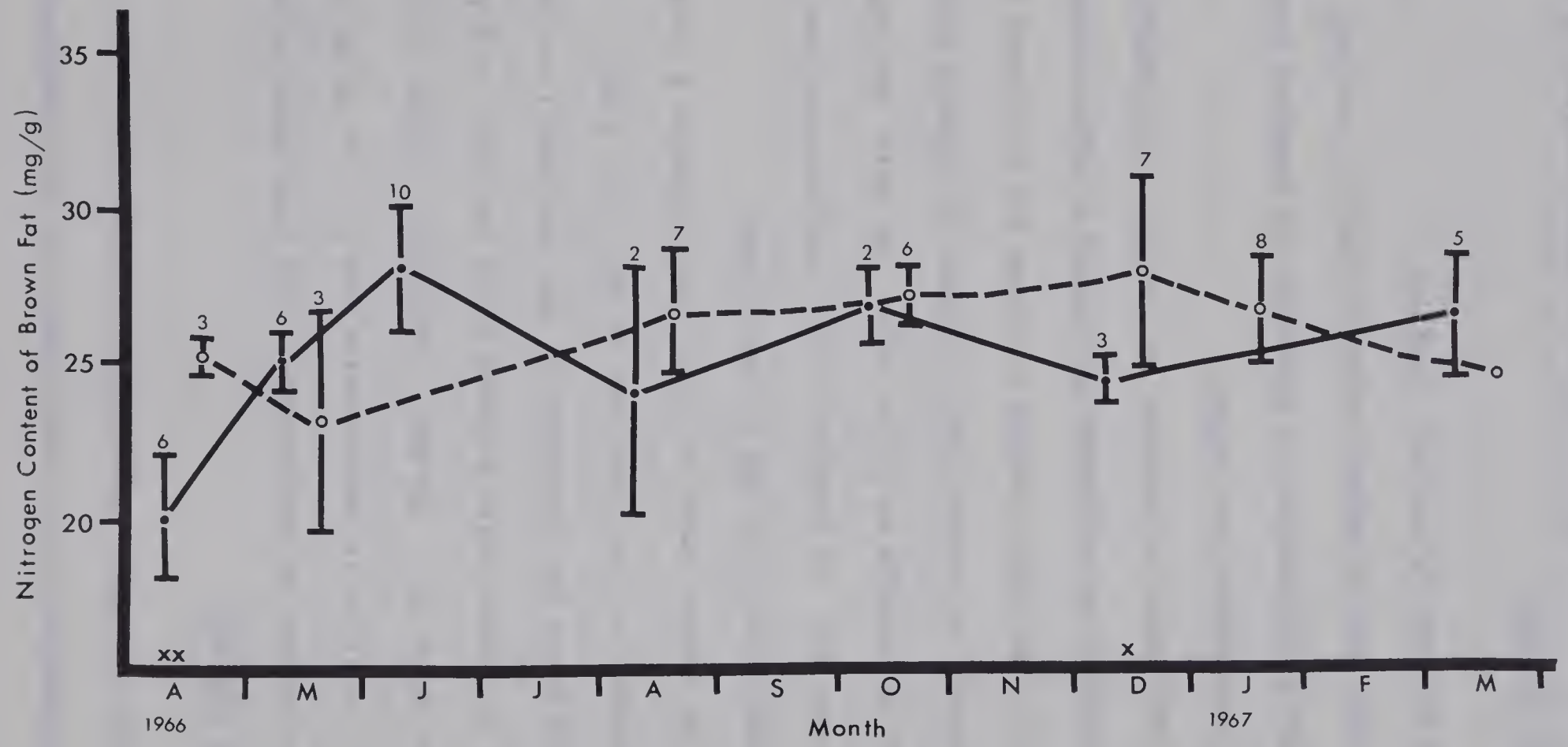
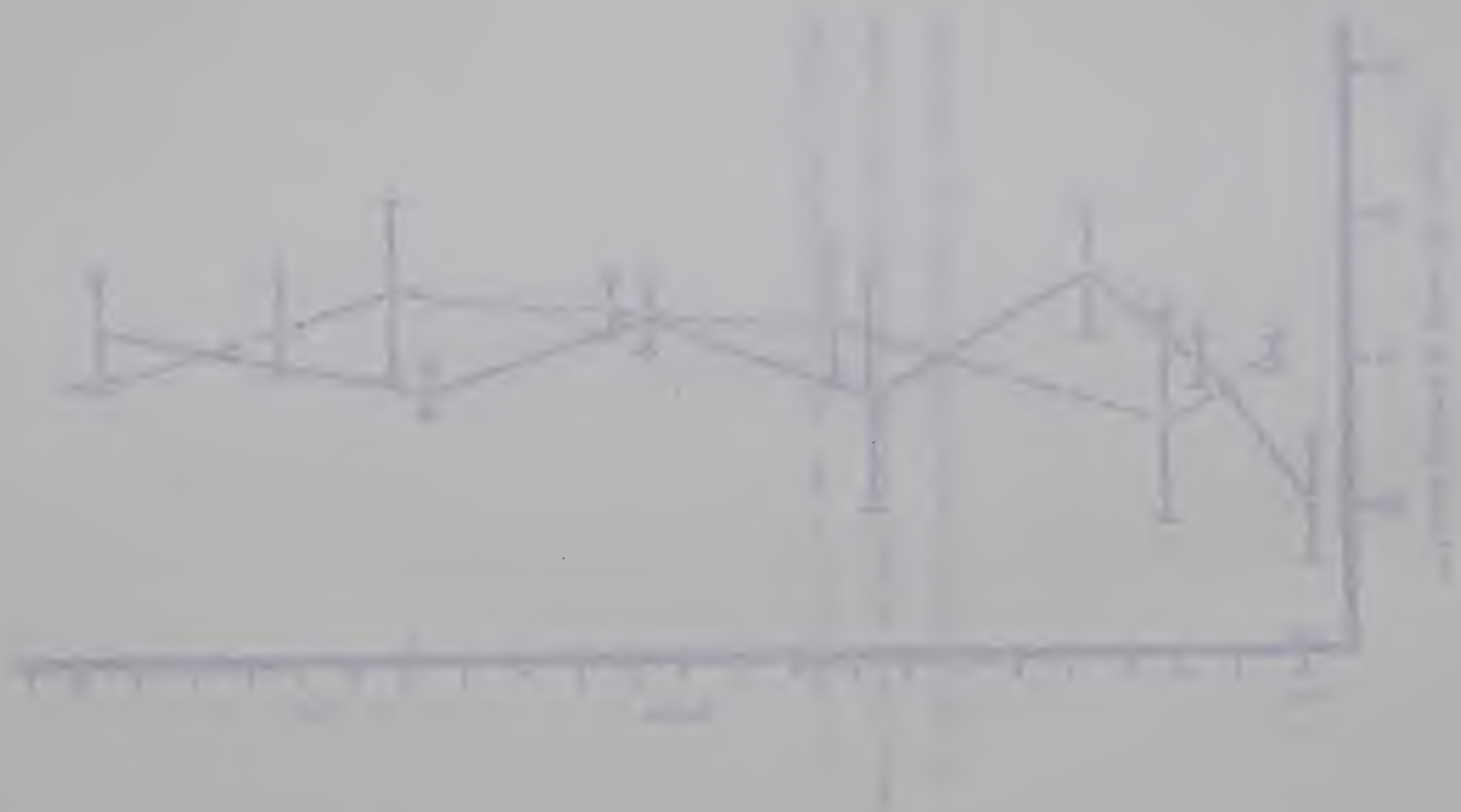

650
600
550
500
450
400
Water Content of Brown Fat (mg/g)
xxx
x
A
M
J
J
A
S
O
N
D
J
F
M
1966
1967
Month

Figure 17. Variations in the nitrogen content of brown fat in mature (solid line) and immature (broken line) voles. Graphic presentation is the same as in Figure 15.


Nitrogen Content of Brown Fat (mg/g)
35
30
25
20
3
6
3
10
6
2
7
2
6
3
7
8
5
xx
x
A
M
J
J
A
S
O
N
D
J
F
M
1966
1967
Month



used to determine if the monthly mean of each component showed significant differences (Appendix Table XII).

a. Lipid Content

The amount of extractable lipid from brown adipose tissue ranged from 26.1 to 41.8% in mature voles and 31.5 to 38.5% in immature voles (Appendix Table IX). The most significant change in lipid concentration occurred in mature voles between April and July (Fig.15). The amount of lipid/g brown fat increased by 60% from April to June ($P < 0.001$) then decreased by 15% from June to July ($P < 0.001$). The remaining sample numbers for mature voles are small but indicate a constant proportion of lipid comprising 34 to 38% of the tissue.

Brown fat, in immature voles that survived the winter of 1965-66, contained an average of 31.5% lipid. By July, new individuals were born and contained an average lipid content of 357mg/g brown fat (Appendix Table IX). The lipid content of brown fat increased significantly ($P < 0.05$) between August and September but showed a highly significant decrease between September and October ($P < 0.001$). No further significant changes occurred throughout the winter.

The lipid content of brown fat of immature voles was significantly higher than that of mature voles in April ($P < 0.005$) and significantly lower in October ($P < 0.001$) (Fig.15).

b. Water Content

The seasonal fluctuations in water content of brown adipose tissue of immature and mature meadow voles (Fig.16) showed the inverse of trends exhibited by the lipid content. The water content of brown adipose tissue

ranged from 41.3 to 60.7% in mature voles and 44.5 to 52.3% in immature voles (Appendix Table X). Significant changes in water content occurred in mature voles between April and July. The water content of brown fat decreased 31.8% between April and June ($P < 0.001$). No significant fluctuations are evident from July to March, and the water content of brown fat ranged from 43.4 to 50.7%.

Brown adipose tissue from immature meadow voles showed a significant decrease in water content between August and September ($P < 0.05$) followed by a significant increase between September and October ($P < 0.005$). A range from 46 to 48% water was maintained in brown fat from October to March (Fig.16).

The water content of brown adipose tissue of immature voles was significantly lower than that of mature voles in April ($P < 0.001$) and significantly higher in October ($P < 0.02$) (Fig.16).

c. Nitrogen Content

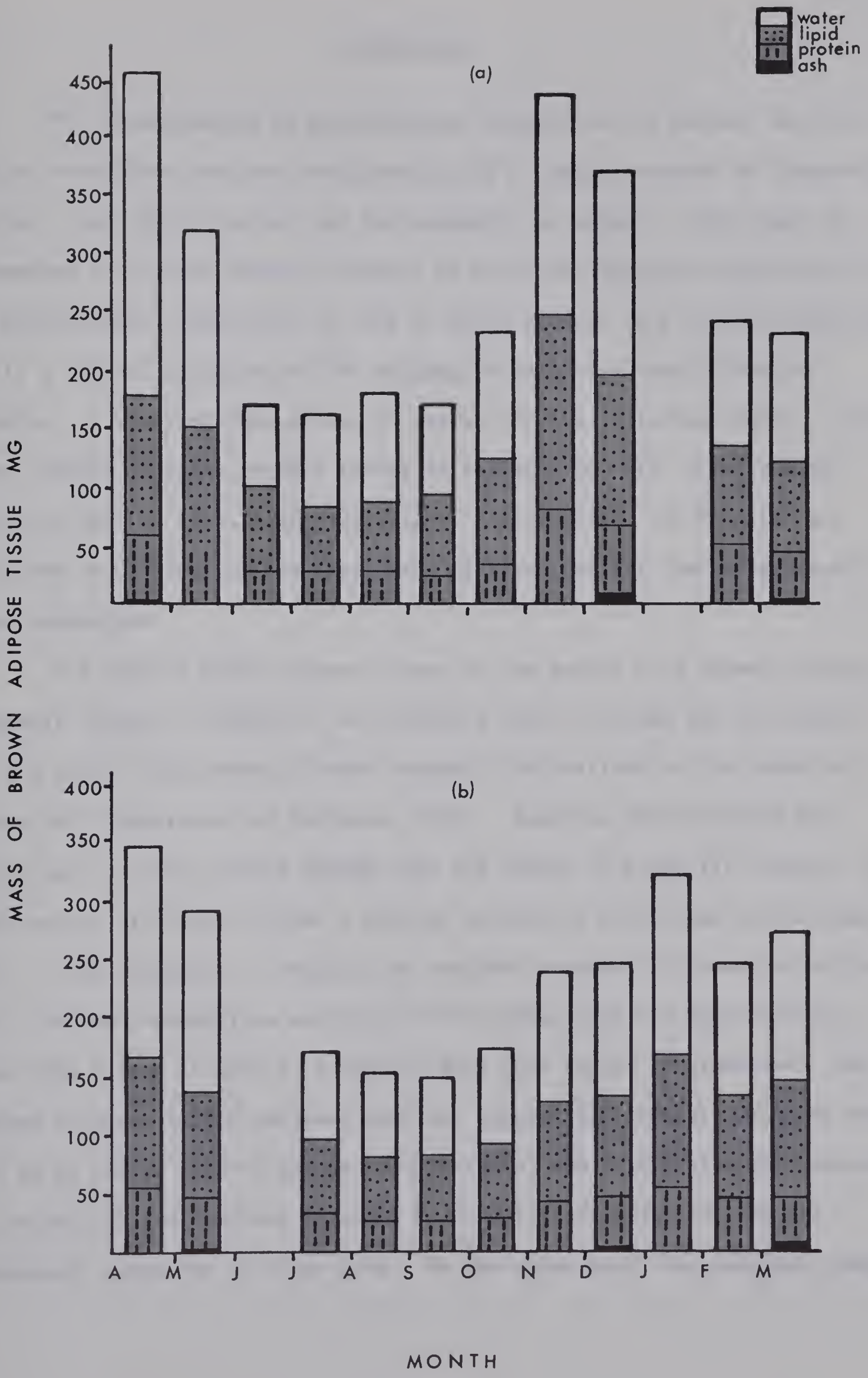
Seasonal changes in the quantity of nitrogen present in brown adipose tissue are plotted in Figure 17. The average amount of nitrogen ranged from 20 to 28mg/g brown fat in mature voles and 23.0 to 27.8mg/g brown fat in immature voles (Appendix Table XI). Between April and June the nitrogen content increased 40% in mature voles ($P < 0.001$). No significant fluctuations occurred in the nitrogen content of immature voles. The brown fat nitrogen of immature voles was significantly higher than that of mature voles in April ($P < 0.005$) and December ($P < 0.05$).

To clarify the compositional data, the average mass of brown

adipose tissue present in the voles each month was proportioned into the following categories: water, lipid, nitrogen, and ash. Nitrogen was converted to protein by multiplying by a factor of 6.25 (assuming the protein contained 16% nitrogen) for the purposes of Figure 18 which shows the proportions of these constituents throughout the year for both mature (a) and immature (b) voles. Figure 18 shows that seasonal changes in the mass of brown fat in mature and immature voles are associated with parallel changes in absolute amount of water, lipid, and protein with occasional alteration of proportions of these constituents.

Figure 18a. Changes in the mass and gross chemical composition of brown adipose tissue in mature meadow voles.

Figure 18b. Changes in the mass and gross chemical composition of brown adipose tissue in immature meadow voles.


water
lipid
protein
ash
(a)
450
400
350
300
250
200
150
100
50
(b)
400
350
300
250
200
150
100
50
A
M
J
J
A
S
O
N
D
J
F
M
MASS OF BROWN ADIPOSE TISSUE MG
MONTH

DISCUSSION

The understanding of physiological adaptations to natural environmental conditions requires consideration of a complex network of integrating factors, both physiological and environmental in nature. This study has attempted to provide further evidence of brown fat having an important role in physiological adaptation to cold by small mammals of a natural population. It is a logical extension of the evidence accumulated from laboratory studies. A study of this nature is useful for the following reason. Aside from looking at brown adipose tissue in response to cold, other natural stresses such as heat, nutrition, light, reproduction, and behavior are involved in determining the importance of brown fat for thermoregulatory heat production.

The mass of brown adipose tissue in the meadow vole showed definite seasonal changes. Similarly, an extensive study, carried out in Poland, on the shrew *Sorex araneus* showed seasonal fluctuations in the amount of brown fat (Buchalczyk and Korybska, 1964). Based on data obtained over three years, these authors showed that the amount of brown fat relative to body weight of shrews follows a similar pattern to that found in the meadow vole. They attempted to explain the seasonal patterns of brown fat on the basis of the reproductive activity of the shrew, with the understanding that this tissue is used as a reserve when food supply is exhausted. More recent evidence (cited earlier) does not support the interpretation of brown fat as an energy reserve *per se*. Furthermore, the interpretations presented by Buchalczyk and Korybska relating brown fat to reproduction are not adequately supported by their data. On the other hand, the seasonal changes

in the mass of brown fat relative to body weight in *M. pennsylvanicus* show an inverse relationship to the environmental temperature (Fig. 10). These findings, plus this author's reinterpretation of the shrew data, provide corroborative evidence that environmental temperature may be a controlling factor which stimulates the physiological mechanisms involved in determining amounts of brown fat. Accordingly, the amount of brown fat is regulated so as to ensure a first line of defence against cold stress at any time of the year. This contribution by brown fat to thermoregulation is, of course, in addition to other adaptive mechanisms to cold such as behavior or insulation. Hayward (1965) showed that behavioral adaptations (use of microclimate, nest building, huddling) were more important than either insulative or metabolic adaptations in a natural population of *Peromyscus*.

Microtus pennsylvanicus, as other microtines, avoid exposure to extreme temperatures by inhabiting a microclimate. Since the ratio of their body mass to surface area is small, relatively large quantities of heat could be lost to the environment. During winter, meadow voles inhabit a subnivean environment where heat conductivity is low (Formozov, 1963). Although these behavioral compensations for thermoregulation are most important relative to the total energy budget of the vole, the same activity occurs outside these conditions, especially during feeding, nest building, and migration. During winter, it was noted in this study that tunnels leading to the snow-air interface were maintained continuously after freshly fallen snow. This activity exposed the mice to the ambient temperature at least for short periods of time. Furthermore, the subnivean microclimate does not exist throughout the year. During the spring and fall

when the snow is absent, the voles may be exposed to relatively cold temperatures for a long period of time. Koshkina (1957) and Fuller (1967) have cautioned against regarding the presence of snow as being entirely beneficial. They point out that a delayed, wet spring, occasioned by fluctuating temperatures, may cause considerable stress and mortality to subnivean occupants. Moreover, fluctuating temperatures vary the degree of thermal conductivity of snow. Heavy wet snow is less effective as an insulator than light fluffy snow (Formozov, 1963). Therefore, at certain times of the year, especially from October to May, there may be considerable demand for thermoregulatory heat production by *M. pennsylvanicus* such that brown adipose tissue would be important.

The validity of the foregoing interpretations is supported by the data in Figure 11. The mean environmental temperature decreases rapidly prior to snowfall and the mass of brown fat relative to body weight increases twofold during this period. The relative proportion of brown fat appears to decrease slightly in immature voles after this prolonged exposure to extreme temperatures (Fig. 11) but continues to increase until mid-winter (Fig. 10). It is clear that brown fat responds during cold stress by an increase in mass. This finding corresponds to that of Roberts and Smith (1967a) for the rat, in which the mass of brown adipose tissue increased after three days' exposure to only 6°C. Furthermore, "atrophy" of brown fat in the spring did not begin until the temperature began to rise in early May (Fig. 10). Such data are most satisfactorily interpreted as evidence that brown fat plays an important role in maintaining homeothermy during "critical periods" in the year when rapid heat production is required.

The percentage of brown fat weight to body weight in the meadow vole is low during the summer; however, it can still serve a thermogenic function.

Donhoffer (1965) showed that brown fat also functions in warm-acclimated animals when they are exposed to temperatures slightly below their thermal neutral zone. Thus, nonshivering thermogenesis (by brown fat) occurs when a slight, extra heat loss occurs, even in non cold-acclimated animals. Therefore, since the mean minimum temperature during the summer did not exceed 12°C (Appendix Table I), which would be well below the thermal neutral zone, a persistent but reduced thermogenic role for brown fat during the summer is indicated. Furthermore, the immature voles maintain a higher level of tissue relative to body weight than the adults during the summer months (Figs. 10,12). This higher proportion of brown fat relative to body weight can be used for the purpose of heat production during early growth when other temperature regulatory mechanisms such as insulation are inadequately developed. The actual mass of brown fat is essentially the same during the summer in immature and mature voles but the proportion to body weight is higher in the young. Thus, a meadow vole born in early summer, and which matures that same summer, does not increase the absolute amount of its brown fat, but the proportion to body weight is lessened. This indicates that the necessity for brown fat to compensate for extra heat lost to the environment is reduced because of the increase in body weight. However, during the colder months, the heat lost to the environment by small mammals would be enhanced and the demand for extra heat production would be greater. In the meadow vole, the relative weight of brown fat increased by twofold in immature voles and fourfold in mature voles as the temperature decreased in the fall (Fig. 10). Furthermore, the mass of brown fat relative to body weight was maintained at a higher level during the winter as compared to that during the summer in both mature and immature voles. This strongly suggests that nonshivering thermogenesis

occurs not only to a greater extent during the winter, but that extra heat production in the meadow vole could be provided largely by brown adipose tissue.

Further evidence on the previous interpretations is required. Lower critical temperatures throughout the season for *M. pennvylsvanicus* should be determined. This would provide information on ambient temperatures required to initiate extra heat production, and some indication of the quantity of heat required at temperatures below the thermal neutral zone. The significance of brown fat for nonshivering thermogenesis in a natural population of voles could then possibly be quantified.

The gross chemical composition of brown adipose tissue in the meadow vole provides some indication that this tissue was metabolized for extra heat production during periods of cold stress. Immature voles showed a significant decrease in the percentage lipid to total mass of tissue in October (Fig.15). The mass of tissue increased, but the absolute amount of lipid remained quite constant (Fig.18b). By November the mass of brown fat continued to increase but the percentage of lipid was restored (Fig.15). Thus it appears that immature meadow voles utilized brown fat lipid in October (prior to snowfall) and then restored it by November. This was not observed in mature voles during this period, and could possibly be explained on the basis of behavioral and/or insulative adaptations causing less heat loss to the environment.

The foregoing data appear to indicate that brown fat cells are capable of restoring any lipid utilized. That is, meadow voles in natural conditions could metabolize brown fat lipid for heat production during periods of cold stress and then restore it during periods when demands for

extra heat production are less severe. Evidence for restoration of brown fat lipid has been shown in the bat following arousals from hibernation. Joel (1965) states that "white adipose tissue seems to be the logical candidate for the major source of lipid for renewal of the brown adipose tissue lipid store following each arousal process". Similarly, white fat surrounding several major brown fat deposits of the meadow vole during the winter (Fig.3a), could serve this purpose. Lipid from food intake could possibly restore brown fat lipid especially during the summer as very little or no white fat is present.

The increased mass of brown adipose tissue in the meadow vole showed increases in the total amount of lipid, water, and protein (Fig.18), and the percentage of each component therein did not change (exception, immature voles in October). The composition of the tissue also remained relatively constant throughout the winter. This indicates that brown fat could possibly be maintained at a compositional level which enables optimal metabolism to occur when extra heat production is required. These findings appear to extend those of Page and Babineau (1950) who found that increase in mass of brown adipose tissue during cold acclimation of rats was due primarily to increases in total lipid-free dry matter and total water of the tissue. The percentage of lipid in brown fat decreased, but the total absolute amount of lipid therein changed very little. To clarify, it has been shown that rats maintained under normal laboratory conditions (temperatures near thermoneutrality) contain a high quantity of brown fat lipid (Chalvardjian, 1964). Similarly, histological examination (Cameron and Smith, 1964) show that brown fat is "infiltrated" with unilocular fat cells. If these animals (containing high lipid content) were acclimated to cold

and the efficiency of brown fat was optimal at a certain compositional level, the increase in mass would be primarily due to water and lipid-free dry matter with little change in absolute amount of lipid.

Conditions in which the absolute amount of lipid accumulates in brown adipose tissue probably do not occur in the meadow vole under natural conditions. Consequently, the mass changes in brown fat are associated with parallel changes in the absolute amount of lipid, water, and protein (Fig.18). Regulation of brown fat cells to maintain sufficient lipid for extra heat production in non-hibernators during periodic exposures to cold requires considerably more study. Furthermore, the composition of brown fat from individual voles should be examined after exposures to fluctuating temperatures in which extra heat production may or may not be required.

The decrease in mass of brown adipose tissue in spring of 1966 also showed parallel changes in the absolute amount of water, lipid, and protein. However, the decreasing mass of brown fat in mature voles appears to be caused primarily by a loss of water, some lipid, and protein (Fig.18). Consequently, the composition of brown fat showed a high percentage of lipid and protein, and a low percentage of water when the mass of tissue was reduced. By July, the composition appears to have readjusted to a required level to enable maximum efficiency for extra heat production. Such changes were not evident in immature voles during this period. However, the composition of brown fat in mature voles showed significant differences from that of immature voles sampled in April, 1966 (Figs. 15, 16,17). This difference cannot be explained on the interpretations of the data suggested thus far. Possibly additional "stress" due to the presence

of large numbers of voles in the area and/or behavioral activity prior to spring breeding is associated with this difference in composition. Nevertheless, the mass of brown fat decreased in both mature and immature voles as the ambient temperature increased in the spring (1966), and the composition of the tissue was associated with decreases in the absolute amount of each component therein. Observations during this period showed a marked reduction in the size of many brown fat deposits, especially in the thoracic region. Whether peripheral cells are reabsorbed by the blood and re-esterfication of lipid in the remaining cells occurs during the "atrophy" of brown fat is unknown and should be studied.

Several interesting findings arose from this study which cannot be explained by existing knowledge of brown fat. For example, females appear to have a significantly higher amount of brown fat than males prior to breeding in spring and early winter. These findings may be related to those of Rothbard (1958) who noticed that brown fat underwent hypertrophy during pregnancy in mice, or those of Ptak (1965), who demonstrated that brown fat is capable of synthetizing steroid hormones. However, the thermogenic role of brown fat in response to temperature change appears to dominate any minor roles brown fat might perform.

Changes in the color of brown adipose tissue throughout the year could not be explained by the gross chemical compositional studies. Heim and Kellermayer(1967) and Joel (1965) found brown fat to be darker in color when depleted in lipid content. This appeared not to be the case in brown fat from the meadow vole. However, a more detailed examination of brown fat tissue from individual voles is required. Changes in diet in spring and fall may produce some changes in lipid components and other biochemical

intermediates present in brown fat.

One of the major problems involved in this study was the different age groups of voles that comprised samples at any one time. Such a problem is unavoidable in a study with the objectives outlined herein. However, the author hopes that by coping with this problem derived from studying a natural population of voles, an additional perspective regarding the physiological role of brown adipose tissue has been obtained.

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APPENDIX

Table I. Official temperatures at Edmonton International Airport from March 1966 to April 1967

Month	Air Temperature °C		
	$\overline{X}$	$\overline{X}$max.	$\overline{X}$min.
March	-4	-1	-10
April	1	6	-4
May	12	19	6
June	14	19	8
July	17	23	12
August	15	20	10
September	14	19	8
October	5	10	0
November	-10	-6	13
December	-10	6	-27
January	-15	-11	-19
February	-10	-3	-15
March	-10	-6	-14

Symbols used: $\overline{X}$ = mean air temperature, $\overline{X}$max. = mean maximum temperature, $\overline{X}$min. = mean minimum temperature.

Table II. Summary of reproductive data on meadow vole.

Month	No. embryos in uteri			No. placental scars		
	$\bar{x}$	Range	n	$\bar{x}$	Range	n
May	4.0	------	2	---	-------	--
June	6.5	4 - 10	19	5.0	------	3
July	6.1	5 - 8	15	6.7	6 - 7	3
August	4.8	4 - 7	5	6.7	5 - 8	3
September	5.8	5 - 7	4	5.4	4 - 7	5
October	6.0	------	1	5.6	3 - 12	11
November	3.0	------	1	5.7	2 - 12	11
December	3.8	2 - 6	15	3.7	3 - 5	3
January	---	------	--	4.0	2 - 7	3
February	---	------	--	---	-----	--
March	4.0	------	1	---	-----	--

Symbols used: n = number of meadow voles observed

$\bar{x}$ = mean value

Table III. Summary of body weights and brown fat masses in immature meadow voles.

Month	Sex	n	$\overline{X}_{b.w.}$ (g)	$\overline{X}_{b.f.w.}$ (g)	B.F. as % B.W. $\overline{x}$	B.F. as % B.W. 2SE
April	male	5	23.6	0.41	1.57	0.32
	female	8	21.3	0.30	1.41	0.16
May	male	5	21.9	0.22	1.01	0.20
	female	10	21.1	0.32	1.55	0.23
June	male	--	----	----	----	----
	female	1	13.7	0.15	1.13	----
July	male	9	17.7	0.17	0.94	0.11
	female	19	17.0	0.17	1.01	0.09
August	male	11	17.1	0.18	1.02	0.19
	female	25	16.2	0.14	0.89	0.07
September	male	14	16.4	0.16	1.03	0.16
	female	24	16.1	0.14	0.91	0.11
October	male	21	17.7	0.17	0.98	0.12
	female	20	17.3	0.17	1.04	0.15
November	male	23	15.5	0.25	1.68	0.22
	female	18	15.4	0.22	1.48	0.15
December	male	9	14.1	0.25	2.09	0.62
	female	8	15.1	0.25	1.68	0.25
January	male	23	14.2	0.32	2.28	0.22
	female	12	13.5	0.31	2.23	0.25
February	male	10	19.6	0.24	1.21	0.29
	female	11	17.2	0.25	1.44	0.14
March	male	4	18.7	0.27	1.48	0.36
	female	3	17.1	0.27	1.56	0.52

Symbols used: $\overline{X}_{b.w.}$ = mean body weight, $\overline{X}_{b.f.w.}$ = mean mass of brown fat, $\overline{x}$ = mean of brown fat as per cent body weight, 2SE = two standard errors of the mean, n = number of sample.

Table IV. Summary of statistical tests (Student's *t*-test) used to compare means of monthly brown fat weights for significant differences.

Months compared	t calc.	d.f.	t table	level of sig.	Interpretation
Immature females					
May/July	3.85	27	3.69	0.001	Significant
July/Aug.	2.57	43	2.42	0.02	Significant
Sept./Oct.	9.09	42	3.55	0.001	Significant
Oct./Nov.	3.85	35	3.60	0.001	Significant
Dec./Jan.	3.39	18	3.20	0.005	Significant
Jan./Feb.	2.90	20	2.84	0.01	Significant
Immature males					
April/May	3.04	8	2.90	0.02	Significant
April/July	4.17	12	3.06	0.01	Significant
Oct./Nov.	4.65	42	3.55	0.001	Significant
Dec./Jan.	3.45	30	3.03	0.005	Significant
Jan./Feb.	4.47	32	3.55	0.001	Significant
Feb./Mar.	2.21	13	2.16	0.05	Significant

Table V. Summary of body weights and brown fat masses in mature meadow voles.

Month	Sex	n	$\overline{X}$ b.w. (g)	$\overline{X}$ b.f.w. (g)	B.F. as % B.W.	
					$\overline{x}$	2SE
April	male	28	33.0	0.47	1.42	0.14
	female	9	29.1	0.42	1.43	0.20
May	male	18	30.2	0.30	0.94	0.13
	female	17	27.7	0.34	1.23	0.13
June	male	30	36.4	0.16	0.46	0.05
	female	19	32.7	0.18	0.56	0.06
July	male	12	36.1	0.18	0.51	0.10
	female	10	37.4	0.14	0.40	0.13
August	male	8	32.0	0.18	0.61	0.11
	female	6	33.1	0.17	0.53	0.11
September	male	1	39.6	0.16	0.41	----
	female	11	32.1	0.17	0.55	0.17
October	male	1	27.8	0.21	0.76	----
	female	8	24.0	0.23	0.99	0.19
November	male	3	26.1	0.38	1.50	0.25
	female	6	22.6	0.29	2.03	0.59
December	male	9	25.8	0.31	1.24	0.08
	female	11	26.5	0.41	1.56	0.20
January	male	0	----	----	----	----
	female	2	16.4	0.36	2.28	0.35
February	male	8	22.5	0.24	1.09	0.17
	female	1	22.4	0.31	1.41	----
March	male	11	24.1	0.21	0.88	0.23
	female	5	21.1	0.27	1.27	0.19
April	male	2	23.4	0.19	0.81	0.58
	female	5	21.1	0.12	0.53	0.64

Symbols used: same as in Table III.

Table VI. Summary of statistical tests (Student's *t*-test) used to compare means of monthly brown fat weights for significant differences.

Months compared	t calc.	d.f.	t table	level of sig.	Interpretation
Mature females					
April/May	2.21	24	2.06	0.05	Significant
May/June	6.69	34	3.65	0.001	Significant
Sept./Nov.	2.22	15	2.13	0.05	Significant
Oct./Dec.	5.49	17	3.97	0.001	Significant
Dec./Mar.	3.66	14	3.33	0.005	Significant
Jan./April	2.81	5	2.57	0.05	Significant
Mature males					
April/May	5.80	44	3.55	0.001	Significant
May/June	7.37	46	3.55	0.001	Significant
Aug./Nov.	9.52	9	4.78	0.001	Significant
Nov./Dec.	2.59	10	2.23	0.05	Significant
Dec./Feb.	3.02	14	2.98	0.01	Significant

Table VII. Summary of statistical tests (Student's *t*-test) used to compare means of monthly body weights for significant differences.

Months compared	t calc.	d.f.	t table	level of sig.	Interpretation
Immature females					
May/July	3.90	27	3.69	0.001	Significant
Jan./Feb.	6.13	21	3.82	0.001	Significant
Immature males					
May/July	3.47	12	3.43	0.005	Significant
Oct/Nov.	2.14	42	2.02	0.05	Significant
Jan./Feb.	6.92	31	3.65	0.001	Significant
Mature females					
May/June	4.00	34	3.65	0.001	Significant
Sept./Nov.	3.75	17	3.22	0.005	Significant
Dec./Mar.	4.58	14	4.14	0.001	Significant
Mature males					
May/June	3.58	46	3.55	0.001	Significant
Aug./Nov.	1.92	9	2.26	0.05	NS

Table VIII. Summary of body weights and brown fat masses of the meadow vole and temperatures prior to snowfall in 1966.

	Air temperature (C)		Body Weight (g)		B.A.T. Weight (g)		
Date	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	n
Oct. 14-16	4.5	-3 - 11	19.6	11.5 - 25.3	0.20	0.12 - 0.31	24
Nov. 3-4	-2.0	-9 - 5.5	15.7	8.0 - 25.8	0.19	0.11 - 0.24	13
Nov. 10-11	-15	-19 - -9	16.2	12.8 - 28.9	0.23	0.19 - 0.32	13
Nov. 18-20	-5.0	-11 - 3	18.0	11.6 - 31.7	0.30	0.17 - 0.44	24
Dec. 3-4	-17	-20 - -12	19.7	14.2 - 39.7	0.25	0.16 - 0.43	9

Table IX. Average lipid content of brown adipose tissue from the meadow vole.

Month	Mature mice			Immature mice		
	n	$\bar{x}$	σ_x	n	$\bar{x}$	σ_x
April	7	261	13	3	316	18
May	7	321	13	3	315	6
June	10	418	31	-	---	--
July	7	355	4	3	357	21
August	3	339	15	7	346	39
September	1	381	--	8	385	20
October	2	380	2	8	346	10
November	2	384	23	8	362	25
December	3	349	3	7	348	28
January	--	---	--	10	354	46
February	1	346	--	5	356	14
March	5	348	17	1	354	--

Symbols used: n = number of samples, $\bar{x}$ = average lipid content (mg/g tissue), σ_x= one standard deviation of the mean.

Table X. Average water content of brown adipose tissue from the meadow vole.

Month	Mature mice			Immature mice		
	n	$\bar{x}$	σ_x	n	$\bar{x}$	σ_x
April	7	607	22	3	523	17
May	7	519	15	3	530	20
June	10	413	34	-	---	--
July	7	488	28	3	471	36
August	3	507	68	7	496	50
September	1	439	--	9	445	28
October	2	448	17	8	482	13
November	2	434	26	8	464	24
December	3	471	10	7	467	36
January	--	---	--	10	471	32
February	1	456	--	5	460	19
March	5	469	25	1	464	--

Symbols used: n = number of samples, $\bar{x}$ = average water content (mg/g tissue), σ_x = one standard deviation of the mean.

Table XI. Average nitrogen content of brown adipose tissue from the meadow vole.

	Mature mice				Immature mice		
Month	n	$\bar{x}$	σ_x	Month	n	$\bar{x}$	σ_x
April	6	20	2.4	April	3	25.3	0.4
May	6	24.9	1.4	May	3	23.0	3.7
June	10	28.1	2.1	August	7	26.6	2.1
August	2	23.8	3.9	October	6	27.0	1.0
October	2	26.7	1.1	December	7	27.8	3.0
December	3	24.3	0.6	January	8	26.6	1.7
March	5	26.4	2.0	March	1	24.4	---

Symbols used: n = number of samples, $\bar{x}$ = average nitrogen content (mg/g tissue), σ_x = one standard deviation of the mean.

Table XII. Summary of statistical tests (Student's *t*-test) used to compare monthly means of lipid, water, and nitrogen content of brown fat for significant differences.

	Months compared	t calc.	d.f.	t table	level of sig.	Interpretation
Lipid Content						
a. Mature voles	Apr./May	8.81	12	4.32	0.001	Significant
	May/June	8.88	15	4.07	0.001	Significant
	June/July	5.69	15	4.07	0.001	Significant
	Aug./Oct.	4.62	3	4.54	0.02	Significant
b. Immature voles	May/July	3.40	4	2.78	0.05	Significant
	Aug./Sept.	2.39	13	2.16	0.05	Significant
	Sept./Oct.	4.84	14	4.14	0.001	Significant
Water Content						
a. Mature voles	Apr./May	8.56	14	4.14	0.001	Significant
	May/June	8.81	15	4.07	0.001	Significant
	Aug./Oct.	3.84	3	3.18	0.05	Significant
b. Immature voles	Aug./Sept.	2.41	14	2.14	0.05	Significant
	Sept./Oct.	3.38	15	3.29	0.005	Significant
Nitrogen Content						
a. Mature voles	Apr./May	4.35	10	3.58	0.005	Significant
	May/June	3.42	14	3.32	0.005	Significant